Title
The Epidemiology of Schistosomiasis Reinfection and Reemergence in Sichuan, China: Implications for Surveillance and Intervention

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The Epidemiology of Schistosomiasis Reinfection and Reemergence in Sichuan, China: Implications for Surveillance and Intervention

by

Elizabeth Jean Carlton

A dissertation submitted in partial satisfaction of the requirements for the degree of Doctor of Philosophy in Environmental Health Sciences in the Graduate Division of the University of California, Berkeley

Committee in charge:
Professor Robert C. Spear, Chair
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Professor Alan Hubbard

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The Epidemiology of Schistosomiasis Reinfection and Reemergence in Sichuan, China: Implications for Surveillance and Intervention

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Abstract

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Professor Robert C. Spear, Chair

Treatment can yield rapid reductions in schistosomiasis infections and morbidity, but sustained control of this water-borne parasitic infection has proven challenging. A better understanding of the epidemiology of schistosomiasis following chemotherapy-based control is urgently needed to inform the design of surveillance systems and interventions in post-control regions.

The research presented here documents the reemergence of schistosomiasis in Sichuan Province, China, evaluates surveillance methods for monitoring schistosomiasis-induced morbidity, using ultrasound, and schistosomiasis reemergence, and examines village-level characteristics that may promote schistosomiasis transmission. Research was conducted in a region where praziquantel-based control was ongoing, using a five-year longitudinal study, and in regions where schistosomiasis reemerged five to fifteen years after human infections were controlled, using a cross-sectional survey of 53 villages.

Reinfection following treatment was common in endemic regions, and widespread human and bovine infections were documented in the reemerging areas. Two commonly used surveillance methods, surveys for Schistosoma japonicum-infected snails and acute schistosomiasis case reporting greatly underestimated the extent of reemergence. Methods that targeted high-risk human populations had high sensitivity. Village-level characteristics that promoted the distribution of S. japonicum eggs, including the use of human waste as an agricultural fertilizer (night soil) and the density of bovines, predicted human infection status. Infection intensities were low in the reemerging region.

Areas where night soil use or bovine ownership are common may be primed for reemergence, providing crucial links in the schistosome life cycle that can promote S. japonicum infections. Interventions and surveillance methods that target populations in high-risk environments may aid in interrupting schistosomiasis transmission and permanently reducing morbidity.
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This research was conducted in close collaboration with the Institute of Parasitic Diseases (IPD) at the Sichuan Center for Disease Control and Prevention. I was fortunate to work with Dr. Qiu Dongchuan, director of IPD, and Dr. Zhong Bo, director of the Sichuan Province Schistosomiasis Control Program. Their knowledge of schistosomiasis and guidance in shaping the studies presented here were invaluable. In addition, I am thankful for the long hours dedicated to field data collection, laboratory analysis and data entry by IPD staff, especially Liu Yang, Zhang Yi and Lu Ding. Thank you to Zhao Lianguo, the director of the Xichang County Anti-Schistosomiasis Control Station, and his staff for making the five-year study described in Chapter 2 possible. While the three counties included in the studies described in Chapters 3 and 4 remain anonymous and therefore, I am unable to thank individuals by name, thanks are also due to the County Schistosomiasis Control Station leaders and staff in each of the three counties for their hard work and hospitality during the extensive field work conducted in 2007.

I benefitted from working with members of the Berkeley Schistosomiasis research team, particularly Drs. Song Liang, Edmund Seto and Justin Remais, as well as Shuo Wang. They have been an excellent source of advice and encouragement. Special thanks are due to Drs. Liang and Spear who encouraged me to study Mandarin. The ability to communicate in Mandarin, at even a basic level, has enriched my experiences in Sichuan.

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The seeds of this dissertation were planted during two years I spent in rural Honduras. There, I saw first hand the terrible toll that infectious diseases extract, particularly in areas where access to health care is limited by a combination of poverty, geography and bureaucracy. Thank you to the United States Peace Corps and my friends in San Jose de la Montaña, Lempira, for allowing me the opportunity to see the potential and the plight of the rural poor. This dissertation is written in their honor.
CHAPTER 1

Introduction

The availability of safe, effective and inexpensive treatments for a number of neglected tropical diseases presents the opportunity to reduce the burden of disease attributable to these infections. Eight diseases – lymphatic filariasis, onchocerciasis, ascariasis, trichuriasis, hookworm infection, trachoma and schistosomiasis – can be treated with a combination of drugs that together cost less than one dollar a day, noting that several drugs are donated by pharmaceutical companies [1]. While the logistics of providing access to such medicines remains a serious obstacle in many areas, the successful implementation of pharmaceutical-based morbidity control programs for these diseases has the potential to yield major improvements in health. Reductions in morbidity can be a first step in a progression of disease control goals. Subsequent goals may include reductions in the incidence of infection (referred to here as infection control) and ultimately, the elimination of transmission. For example, efforts to eliminate blindness due to onchocerciasis have been so successful that the feasibility of elimination of the parasite in endemic regions in Africa as well as the Americas is being considered [2-4]. Pharmaceutical-based reductions in morbidity mark an important step forward in disease control efforts. But the transition to infection control and elimination presents a new set of challenges.

Following reductions in infection prevalence and morbidity, the risk of disease reemergence looms large. The dynamics of transmission may be altered as infections concentrate in high risk populations and areas, including those missed by morbidity control programs. When parasite populations are limited, identifying locations where infections remain or have returned can be challenging, and may require surveillance strategies tailored for low-prevalence situations. In addition, many neglected tropical diseases are transmitted by parasites whose survival is sensitive to a number of environmental variables. Where parasite populations are low, environmental conditions that promote continued transmission may modulate the long term success of disease control programs. Recent achievements and setbacks in the control of schistosomiasis in China offer the opportunity to better understand the dynamics of transmission following morbidity control. This dissertation offers a detailed examination of human schistosomiasis in southwest China following chemotherapy-based control efforts and explores the implications of the situation in China for schistosomiasis control elsewhere and the control of other neglected tropical diseases.

An estimated 207 million people are infected with schistosomiasis worldwide [5]. Infections are largely due to three main species of schistosomes: *Schistosoma mansoni*, found in Africa, the Middle East, South America and the Caribbean, *S. japonicum* found in China, the Philippines and, to a limited extent, Indonesia, and *S. haematobium*, found in Africa and the Middle East. Infections occur when people contact fresh water contaminated with cercaria, a
free-living larval stage of the parasite capable of penetrating human skin (Figure 1). Once inside the human host, the parasite migrates through the lungs to the portal veins where it matures into an adult worm and mates. The intestinal schistosomes, *S. japonicum* and *S. mansoni*, settle into the mesenteric blood vessels (*S. japonicum* adult worms also inhabit the superior hemorrhoidal veins that drain the large intestine) and commence egg laying, typically four to six weeks after infection [6]. Eggs not trapped in host tissues are excreted in stool. The adult *S. haematobium* worm pairs reside in the blood vessels surrounding the bladder and ureters, and eggs are excreted in urine. Schistosome eggs hatch upon contact with fresh water, releasing miracidia. This free-living larval stage infects the aquatic or, in the case of *S. japonicum*, the skin of other mammalian hosts, shed their tails and become schistosomula. Schistosomula migrate through the lungs to the portal vessels where they mature into adult worms and mate.

Adapted from http://www.dpd.cdc.gov/dpdx/html/schistosomiasis.htm
While the burden of disease due to schistosomiasis is primarily due to chronic morbidity, the number of schistosomiasis deaths from gastrointestinal bleeding and renal failure may exceed 200,000 annually in Sub-Saharan Africa [14]. *S. haematobium* has been classified as a group one carcinogen by the International Agency for Research on Cancer for its ability to cause bladder cancer [15,16]. *S. japonicum* and *S. mansoni* have been associated with liver and colorectal cancers however the evidence to date is inconclusive [17-20]. Schistosomiasis accounts for an estimated 1.7 million Disability Adjusted Life Years, but this measure excludes the impacts of anemia and malignancies and likely greatly underestimates the true burden of disease [12,21].

Schistosomiasis can be treated with praziquantel, a safe, effective and now inexpensive drug that was introduced in the 1970s. A single dose of praziquantel, which targets the adult worm, is effective against all major schistosomes, curing 70 – 100% of infections [7,22,23]. Side effects are minor and transient, rarely lasting more than 24 hours, and include headache, nausea, dizziness and abdominal pain [7,22,24]. In contrast, the antischistosomials that preceded praziquantel, including several trivalent antimonials, had severe side effects including, in some cases, cardiac arrhythmias and an anaphylaxis-like shock, both of which could be fatal [25]. Today, an average dose of praziquantel costs $0.20 to $0.30 in endemic countries [26]. Because of its low cost, single-dose administration, high cure rates and excellent safety profile, praziquantel is appropriate for mass chemotherapy in endemic areas. But praziquantel does not protect against future infection, and reinfection following treatment is common. To date, there is no vaccine for schistosomiasis, although it is an area of active research [27].

With the advent of a safe and inexpensive treatment for schistosomiasis, efforts to reduce morbidity through distribution of praziquantel have been launched by governments, multinational banks and non-governmental organizations. In 1992, China and the World Bank jointly began a $150 million, ten-year initiative to reduce schistosomiasis in China, primarily through the distribution of praziquantel. Annual mass drug distribution was conducted in highly endemic areas where infection prevalence exceeded 15%. In areas with lower infection prevalence, biannual testing was conducted to target treatment directly to infected individuals. In addition, bovines were treated and snail populations controlled through the application of molluscicide. Five years into the program, infection prevalence had been halved. By the end of the program in 2002, infection prevalence was below 1% in over 50% of participating counties [28]. Today, China is looking past morbidity control at country-wide schistosomiasis elimination. Current goals include the reduction of infection prevalence below 0.1% in most endemic areas by 2015 [29].

In the wake of China’s morbidity control program, the World Health Organization adopted a resolution in 2001 that at least 75% of children in schistosomiasis-endemic areas should be treated by 2010 [30]. The following year, the Schistosomiasis Control Initiative (SCI) was launched in sub-Saharan Africa with a $30 million dollar grant from the Bill and Melinda Gates Foundation in order to reduce schistosomiasis morbidity in school children. Six countries were selected to roll out national schistosomiasis control programs that emphasized preventative chemotherapy, the mass distribution of praziquantel (and the anti-helminthic abendazole which treats soil-transmitted helminthiases), primarily to school children [31,32]. Early evaluations have shown sharp reductions in schistosomiasis infection prevalence and intensity following chemotherapy, as well as declines in anemia [33,34].

As has occurred in China, chemotherapy-based control programs can usher in the next phase of disease control, one that emphasizes reductions in the incidence of infections and
ultimately, the elimination of transmission [35]. This transition marks laudable progress in schistosomiasis control. But recent experiences warn that reductions in infection and morbidity may be transient. Following cessation of the World Bank loan project in China, infection prevalence rose in the remaining endemic areas and new infections were detected in areas where transmission was thought to have ceased [29,36]. In West Africa, large reductions in infection prevalence and intensity following SCI-funded chemotherapy were followed by a rise in infections in some regions [37]. These setbacks underscore the need to better understand the dynamics of transmission following pharmaceutical-based control efforts so that reemergence may be avoided and improvements in health sustained.

In the following chapters, schistosomiasis morbidity, surveillance and the environmental determinants of infection are examined in two regions where chemotherapy has been used to control schistosomiasis. The research takes place in Sichuan province, located in southwest China, where schistosomiasis has historically been endemic in rural areas with irrigated agriculture. It is my goal to provide a detailed look at schistosomiasis transmission in the post-control period. Recognizing that new tools may be needed for tracking disease, I examine methods for morbidity and infection surveillance. I also document the reemergence of infection in previously endemic areas and seek to identify environmental determinants of transmission in areas where prevalence is low.

In Chapter 2, I evaluate the use of ultrasonography, a non-invasive method, to assess schistosomiasis-induced liver damage and document changes in morbidity following treatment. Portable ultrasound machines allow for field-based measures of liver damage and can enable the evaluation of disease control efforts on sub-clinical morbidities. Ultrasound is considered the “gold standard” for documenting fibrosis due to *S. mansoni* and *S. haematobium* [38], but uncertainties remain about the value of ultrasound measures of *S. japonicum* morbidity. The research takes place in Xichang County, where schistosomiasis transmission was ongoing and infection intensities were high at the initiation of the five-year longitudinal study. Praziquantel-based control efforts were implemented as part of the study, including testing and treatment of all infections. I discuss the suitability of ultrasound for documenting *S. japonicum* morbidity and the impact of praziquantel-based control on ultrasound-detected morbidity.

The research described in Chapters 3 and 4 takes place in three counties where schistosomiasis reemerged five to fifteen years after Chinese transmission control criteria were met, a phenomenon first documented by Liang et al., primarily through the discovery of acute cases of schistosomiasis [36]. In Chapter 3, I document the distribution of infections in humans, non-mammalian reservoirs and the intermediate snail host in 53 villages following the reemergence of schistosomiasis in the region. The work offers an in-depth look at schistosomiasis transmission following near elimination of the parasite. I also evaluate methods for detecting human schistosomiasis in this post-control region, as reduced infection intensities may alter the performance of traditional surveillance methods.

In Chapter 4, I examine the impact of environmental conditions on human infection risk and estimate the impact of environmental interventions on infection prevalence. I describe both household- and village-level characteristics that might promote or impede the distribution of schistosome eggs, including sanitation, the use of human waste as an agricultural fertilizer and the density of non-human reservoir populations. This analysis ultimately examines whether high risk environments for schistosomiasis transmission, particularly in post-control regions, can be identified. The ability to identify such locations can enable targeted surveillance and interventions to reduce the risk of reinfection and reemergence.
Finally, the implications of the findings presented here for schistosomiasis control in China and globally, as well as the implications for other environmentally mediated infections, are discussed in Chapter 5.

The research presented here was conducted in close collaboration with the Institute of Parasitic Diseases (IPD) at the Sichuan Center for Disease Control and Prevention and the schistosomiasis research team at the University of California, Berkeley (UCB) led by Dr. Robert Spear. The nearly two-decade long relationship between IPD and UCB has been fostered by many scientists on both sides of the Pacific whose work has preceded mine. Undoubtedly, the examination of the politically sensitive issue of schistosomiasis reemergence in areas that had previously attained control would not have been possible without the mutual trust that has been built since the early 1990s. Dr. Song Liang, in particular, was instrumental in working with IPD scientists and county anti-schistosomiasis control stations to document county-level evidence of reemergence in Sichuan province. In addition, the data collected in Xichang County, described in Chapter 2, and in over 50 reemerging villages, described in Chapters 3 and 4, were collected by capable IPD staff. Dr. Zhong Bo, director of the Schistosomiasis Control program at IPD, enabled the refinement of practical, culturally-appropriate data collection instruments and mobilized her staff to collect and process thousands of biological samples to assess *S. japonicum* infections and administer thousands of questionnaires while adhering to rigorous quality control standards. Dr. Qiu Dongchuan, director of IPD, made possible the work presented in Chapter 2 by having the foresight to collect morbidity data from the initiation of a study of endemic schistosomiasis transmission in Xichang County.

The years of research conducted by UCB and IPD teams have also laid the foundations for the questions I explore here. Much of this research has focused on deterministic mathematical modeling of endemic schistosomiasis transmission [39]. These models integrate biological and environmental parameters that have been estimated through the thoughtful investigations of UCB and IPD scientists, including studies of human water contact patterns [40,41], snail population dynamics [42] and cercarial transport [43-45]. Mathematical modeling has provided proof-of-concept that environmental conditions can impact the long-term success of praziquantel-based schistosomiasis control programs in endemic areas [46,47]. This has motivated efforts to identify high-risk environments for schistosomiasis transmission, including my exploration of schistosomiasis reemergence and the role of environmental conditions in promoting infections in previously controlled areas. I have used epidemiological and statistical methods to characterize schistosomiasis infection patterns in reemerging areas. Ideally, the results of mechanistic mathematical models inform the questions addressed by data-driven statistical models, which can lead to the refinement of mathematical models, leading to a more robust understanding of the drivers of human schistosomiasis and ultimately, its successful control. Efforts to create a mathematical model of schistosomiasis reemergence will follow and hopefully be informed by the work presented here.

Ultimately, long-term improvements in the health of populations that are at risk of schistosomiasis and other neglected tropical diseases hinge on the ability to motivate governments and donors to allocate resources towards morbidity reduction, and to look beyond the logistics of drug distribution to consider what it will take to sustain control. The work presented here offers a glimpse of the challenges and opportunities presented by efforts to tackle neglected tropical diseases through pharmaceutical-based morbidity control. Ideally, public health programs yield rapid reductions in severe morbidity and promote the long-term health of populations. Much attention has been focused on the former, and justifiably so, as today many
populations at risk of schistosomiasis still do not have even basic access to praziquantel. Nonetheless, populations impacted by schistosomiasis deserve not only rapid treatment plans, but public health programming that ensures long term protection from schistosomiasis-induced morbidity and mortality.

References

CHAPTER 2

Evaluating the use of ultrasound to document morbidity from *Schistosoma japonicum* infection and the impact of treatment: a five year cohort study in Southwest China

Introduction

Schistosomiasis causes morbidity in the human host through the schistosome egg, which triggers inflammation and fibrosis that can lead to anemia, impaired growth and in severe cases, gastrointestinal bleeding and death [1-4]. The major intestinal schistosomes, *S. japonicum*, found in Asia, and *S. mansoni*, found in Africa, the Americas and the Middle East, mature, mate and lay eggs in the portal and mesenteric blood vessels. Eggs are transported to the liver where they are encapsulated and the granulomas that form induce an inflammatory cascade that includes the deposition of collagen and extracellular matrix proteins, a normal liver repair process that can lead to fibrosis when fibrogenesis exceeds the replacement of scar tissue with healthy cells [5,6]. The immune regulation of this process is currently being explored [7].

Approximately 700,000 people are infected with *S. japonicum* in China [8]. As in other parts of the world, schistosomiasis control efforts have focused primarily on the distribution of the antischistosomal drug, praziquantel [9,10]. However the success of such efforts hinges on the ability to reduce not only schistosomiasis infections, but also morbidity. A means of documenting *S. japonicum* morbidity is essential to the evaluation of disease control efforts [11].

Ultrasound is a non-invasive method that can be used to evaluate pathologies of the liver and spleen resulting from schistosomiasis infection. Fibrosis along the portal vein and its branches produces a clay pipestem pattern, as the portal tracks are lined with fibrous tissue, and is observed following both *S. mansoni* and *S. japonicum* infection. This periportal fibrosis can be assessed qualitatively through image classification [12] or quantitatively by measuring the diameter of three secondary portal branches [13]. Unique to *S. japonicum* infection is parenchymal fibrosis, a network pattern that is often described as fish scale or tortoise shell-like. Parenchymal fibrosis is likely due to the smaller *S. japonicum* egg size which allows the parasite eggs to enter smaller portal veins and reach a greater portion of the liver [7]. *S. japonicum* adult females produce ten times more eggs per day than *S. mansoni*, and the eggs often are deposited in clusters, two additional factors that exacerbate the severity of *S. japonicum* morbidity relative to other schistosome species and may contribute to the unique fibrotic pattern [3]. Ultrasound can also be used to assess hepatomegaly, splenomegaly and dilation of the portal vein, all of

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1 A similar version of this manuscript has been published: Carlton EJ, Hsiang M, Zhang Y, Johnson S, Hubbard A, Spear RC [In press] The Impact of *Schistosoma japonicum* Infection and Treatment on Ultrasound-Detectable Morbidity: A Five-Year Cohort Study in Southwest China. PLoS Neglected Tropical Diseases.
which result from portal hypertension. Recognizing the potential of ultrasound to be used to evaluate the impact of disease control efforts, draft protocols for the use of ultrasound in assessing schistosomiasis morbidity were established for each of the three major schistosome species in 1990 [13,14]. Follow-up meetings to refine the protocols for *S. haematobium* and *S. mansoni* were held in 1996 and 1997 [15]. Ultrasound is considered the gold standard for schistosomiasis morbidity assessment for these species [11]. However, due to insufficient evidence, the protocol for *S. japonicum* has not been revised.

To date, a comprehensive evaluation of the *S. japonicum* ultrasound measures proposed in Cairo, including their relationship to *S. japonicum* infection and the way in which they change following treatment, is lacking. Li et al. [16,17] offer the most complete examination of the proposed ultrasound measures to date, tracking a highly exposed cohort over five years, but as standard organ sizes have only recently been published [18], assessments of liver and spleen enlargement did not account for participant height as currently recommended [15]. Failure to account for height-specific variation in organ sizes can lead to underestimates of morbidity, particularly in pediatric populations [19]. Ideally, ultrasound measures are associated with *S. japonicum* infection and decline following treatment [13]. The available data are conflicting on both criteria. Treatment has yielded declines in parenchymal and periportal fibrosis in a highly exposed Chinese cohort, but 68% of participants experienced no change in parenchymal fibrosis over a five-year period [16,17], and other studies have not found significant declines in periportal fibrosis following *S. japonicum* treatment [20,21]. Further, direct relationships between infection and fibrosis or measures of portal hypertension have not been demonstrated consistently [16,17,22].

Here, 578 individuals were followed over five years in order to examine the relationship between *S. japonicum* infection and five liver ultrasound measurements recommended in the draft protocol as well as two spleen ultrasound measurements included in the standard Chinese examination. I hypothesized that *S. japonicum* infection is associated with ultrasound-detected measures of hepatic fibrosis and that treatment of infected individuals leads to declines in ultrasound-detected morbidity.

**Methods**

Participants were drawn from a cross-sectional survey in 2000 that revealed high infection prevalence and intensity (ranging from 3% to 73%) in 20 villages distributed in the hilly terrain of Sichuan Province in Southwest China, where irrigated agriculture for the cultivation of rice, wheat, corn and tobacco is the primary source of *S. japonicum* infection [23]. As part of the survey, all residents age 4 to 60 years in 20 villages in Xichang County, Sichuan Province, China were invited to be tested for *S. japonicum* infection and to answer a brief questionnaire. In this study, a random number generator was used to select approximately 30% of the population, stratified by village and occupation, for ultrasound examination. Ten villages with high infection prevalence and intensity in 2000 were selected to be followed longitudinally using infection surveys and ultrasound examinations over the next five years. Participants in the cohort presented here include individuals who had an ultrasound examination in 2000 and lived in one of the ten villages followed through 2005.

All participants provided oral informed consent and those with *S. japonicum* positive stool examinations were provided treatment. As the survey procedures used in this study are the same as those used by the Institute of Parasitic Diseases, Sichuan Center for Disease Control and
Prevention (IPD) for schistosomiasis surveillance and given the high rates of illiteracy in the population, oral informed consent was used and documented by IPD staff. The research protocol and consent procedures were approved by the Sichuan Institutional Review Board and the University of California, Berkeley, Committee for the Protection of Human Subjects.

**Ultrasound examination.** Ultrasound examinations were conducted in fall 2000, 2002 and 2005. All examinations were conducted by one trained ultrasonographer using a single portable ultrasound machine (Hitachi EUB 405, Hitachi Corporation, Tokyo, Japan) and 3.5 MHz probe (Hitachi EUP-C314T, Hitachi Corporation, Tokyo, Japan). Participants were examined in the supine position at a central location in each village. The ultrasonographer was blind to infection status.

Liver ultrasonography was conducted according to the 1990 draft guidelines [13,14]. Liver parenchymal fibrosis was graded 1 through 3 based on observed lesions, or 0 if none were present. Periportal fibrosis was assessed by grading the average diameter, from outer wall to outer wall, of three peripheral branches of the portal vein between the first and third branching point (grade 0: <3mm; grade 1: 3 to 5 mm; grade 2: >5 to 7 mm; grade 3: >7 mm). As has been done previously, grades 0 and 1 were combined for analysis [16]. The internal diameter of the portal vein was measured at the entry point of the portal vein into the liver. The length of the left liver lobe was measured in a longitudinal section along the left parasternal line, and the length of the right liver lobe was measured as the maximum oblique diameter using a right anterior axillary view according to the revised guidelines established for *S. mansoni* [15].

Two measurements of the spleen that are part of the standard Chinese examination were also included: spleen thickness, measured from the hilum to the opposite section, and the internal diameter of the spleen vein, measured at the entry point to the spleen [24].

Because organ and vein sizes vary with height, left and right liver lobe length, portal vein diameter and spleen thickness were evaluated using height-specific standard sizes drawn from a Chinese population where schistosomiasis is not endemic [18]. Measurements greater than two standard deviations above the mean size for height were classified as abnormal. Height was measured in 2002 in 440 of the 578 cohort members. The 343 adults (≥18 years old in 2000) with height measurements were assigned their 2002 height throughout the study. Children (<18 years old in 2000) were measured again in 2007 as part of a study described elsewhere [19]. The heights measured in 2002 and 2007 from 119 children (60 with one height measurement, 59 with two height measurements) were used to generate an age- and sex-dependent random intercept model in order to impute heights during the years children weren’t measured. Height for child *i* at time *j* was calculated using the following equation:

\[
H_{ij} = 0.604 + 0.100A_{ij} - 0.003A_{ij}^2 - 0.109S_i + 0.012A_{ij}S_i + \zeta_i
\]

where *A*<sub>*ij*</sub> represents a child’s age at time *j*, *S*<sub>*i*</sub> represents his or her sex (*S* = 1 for males) and \(\zeta_i\) represents his or her random intercept. The model was fit using xtmixed in Stata 10.1 (StataCorp, College Station, TX, USA) and \(\zeta_i\) was predicted using empirical Bayes [25]. As random intercept models assume parametric distribution of residuals, first and second order residuals were examined and were normally distributed. In order to assess model fit, predicted heights were regressed against observed heights, and \(r^2 = 0.973\) (Figure 1). Organ and vessel measures could not be height adjusted for the 86 adults and 26 children without height measurements or for the four individuals whose age was missing. Participants with and without height data did
Participants were tested for infection with *S. japonicum* in the fall of 2000 and 2002 using the miracidial hatch test [26] and the Kato-Katz thick smear procedure [27]. For each hatch test, a stool sample weighing at least 30 g was suspended in aqueous solution, filtered using copper mesh to remove large particles (40-60 mesh), followed by nylon gauze (260 mesh) to concentrate schistosome eggs. This sediment was re-suspended with distilled water in a 250 ml Erlenmeyer flask. Flasks were examined for miracidia 30 to 60 minutes, four hours and eight hours after suspension if temperatures were above 30 degrees Celsius, or at 6, 12 and 18 hours at lower ambient temperatures. In 2000, three miracidial hatch tests were conducted per person using stool samples from three distinct days. In 2002, due to logistical constraints, one miracidial hatch test was conducted per person. The Kato-Katz protocol was identical both years: three 41.5 mg slides were prepared from one homogenized stool sample in 2000 and 2002. Infection intensity, in eggs per gram of stool (EPG), was calculated as the total number of *S. japonicum* eggs present on the slides divided by the total sample weight. Participants were classified as infected if at least one test was positive. Everyone testing positive for *S. japonicum* was provided praziquantel treatment by the county Anti-Schistosomiasis Control Station. In addition, praziquantel was administered to all residents in the study villages in 2003, as part of a nation-wide effort to control infectious diseases following the outbreak of severe acute respiratory syndrome.

Participant age, sex, occupation and highest level of schooling were obtained by interview in fall 2000.

**Statistical analysis.** In order to assess whether the participants in the ultrasound cohort were representative of the village populations from which they were selected, cohort participants were compared in terms of age, sex, occupation, educational attainment and 2000 *S. japonicum* infection status to individuals who participated only in the cross-sectional demographic and

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**Figure 1.** A comparison of measured heights and heights predicted using an age and sex dependent random intercept model for children less than 18 years old in 2000, $r^2 = 0.973$. 

![Graph showing predicted vs. actual heights](image)
Figure 2. Directed acyclic graph illustrating a proposed model of the relationship between *S. japonicum* infection and ultrasound-detected morbidity, accounting for potential confounders. Age, sex and year can predict infection and may independently affect ultrasound-detected morbidities and therefore are considered confounders. Occupation, village and education are not confounders in this model as they may cause infection but are not independently associated with morbidity.

Liver parenchymal fibrosis grade is an ordinal measure and was examined using ordinal logistic regression, a population averaged model, using a sandwich type estimator for inference accounting for within-subject residual correlation [28]. Because ordinal logistic regression assumes the effect of a predictor on an outcome is constant for each stepwise increase in the outcome, the Brant test was used to check that this parallel regression assumption was not violated [29]. For all other liver and spleen measures and for predictors of *S. japonicum* infection status, generalized estimating equation (GEE) logistic regression with exchangeable correlation was conducted [30]. The Huber/White/sandwich estimator of variance was used, an estimator that is robust to misspecification of the outcome distribution [31,32].

The relationship between *S. japonicum* infection and ultrasound-detected abnormalities was first assessed by examining the impact of current infection status and, separately, infection intensity, on current ultrasound measures. Because infection intensity was highly right-skewed, it was categorized into approximate quartiles. In order to examine the role of past infection on current morbidity, the relationship between infection status and intensity two to three years prior to the ultrasound examination and ultrasound-detected abnormalities was also examined (for example: 2000 infection status as a predictor of ultrasound-detected morbidity in 2002, and 2002 infection status as a predictor of ultrasound-detected morbidity in 2005). I hypothesized that age, sex and year of examination could modify the effect of infection on morbidity, so each model was run including all possible first-order interaction terms. The Wald test was used to test for significant interactions and, if present, terms were removed from the model step-wise until only interaction terms significant at p-values <0.05 remained. In the absence of effect modification, these same variables could confound the relationship between infection and ultrasound-detected morbidity. While occupation, village and educational status were considered potential predictors of *S. japonicum* infection, they are unlikely to affect ultrasound morbidity independent of infection status. As they do not fit the definition of confounders [33], they were not controlled for in models examining the relationship between infection and ultrasound-detected abnormalities (Figure 2). The change in ultrasound-detected abnormalities over time was examined adjusting for age and sex.
Table 1. Characteristics of residents in the ten study villages, including 578 ultrasound participants and 1,333 people who participated only in the 2000 infection or demographic surveys.

<table>
<thead>
<tr>
<th></th>
<th>Ultrasound participants</th>
<th>Other village residents</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.  (%)</td>
<td>No.  (%)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>295 (51)</td>
<td>634 (48)</td>
</tr>
<tr>
<td>Male</td>
<td>279 (49)</td>
<td>698 (52)</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;18</td>
<td>145 (25)</td>
<td>423 (32)</td>
</tr>
<tr>
<td>18-29</td>
<td>141 (25)</td>
<td>281 (21)</td>
</tr>
<tr>
<td>30-39</td>
<td>130 (23)</td>
<td>298 (22)</td>
</tr>
<tr>
<td>40-49</td>
<td>95 (17)</td>
<td>186 (14)</td>
</tr>
<tr>
<td>50+</td>
<td>63 (11)</td>
<td>145 (11)</td>
</tr>
<tr>
<td>Occupation*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not farmer</td>
<td>37 (9)</td>
<td>72 (8)</td>
</tr>
<tr>
<td>Farmer</td>
<td>392 (91)</td>
<td>833 (92)</td>
</tr>
<tr>
<td>Education*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elementary school or less</td>
<td>253 (59)</td>
<td>554 (61)</td>
</tr>
<tr>
<td>Some middle school or higher</td>
<td>175 (41)</td>
<td>348 (39)</td>
</tr>
<tr>
<td>Infected with S. japonicum in 2000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>299 (53)</td>
<td>657 (53)</td>
</tr>
<tr>
<td>Yes</td>
<td>265 (47)</td>
<td>580 (47)</td>
</tr>
<tr>
<td>S. japonicum infection intensity in 2000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 EPG</td>
<td>333 (59)</td>
<td>730 (59)</td>
</tr>
<tr>
<td>1 - 10 EPG</td>
<td>60 (11)</td>
<td>106 (9)</td>
</tr>
<tr>
<td>11 - 50 EPG</td>
<td>81 (14)</td>
<td>205 (17)</td>
</tr>
<tr>
<td>&gt;50 EPG</td>
<td>89 (16)</td>
<td>188 (15)</td>
</tr>
</tbody>
</table>

*Includes adults (≥18) only.

Age was modeled as a categorical variable when examining the relationship between age and ultrasound-detected morbidity to allow a non-linear relationship. Because liver abnormalities increase with age, age was treated as a continuous variable when included as a confounder in models testing the relationship between infection and morbidity, or changes in morbidity over time. Periportal fibrosis grade was modeled as a binary variable, as grades 0 and 1 were combined and no grade 3 fibrosis was detected. Tests for trend were calculated by treating categorical variables as ordinal. All results were assessed for statistical significance setting $\alpha = 0.05$. Statistical analyses were conducted using Stata 10.1 (StataCorp, College Station, TX, USA).

Results

In 2000, 578 people from ten villages were examined using ultrasound. Participants were similar to the 1,333 residents in these villages who participated in infection and demographic surveys only (Table 1). The mean age of ultrasound participants was 29.8 years (range 4 – 61 years).
Table 2. The prevalence and odds of *S. japonicum* infection by age, sex, occupation, educational attainment, year and village.

<table>
<thead>
<tr>
<th></th>
<th>Tested</th>
<th>Infected (%)</th>
<th>Tested</th>
<th>Infected (%)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Year</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Year 2000</td>
<td>564</td>
<td>265 (47)</td>
<td></td>
<td></td>
<td>1.00</td>
</tr>
<tr>
<td>Year 2002</td>
<td>504</td>
<td>161 (32)</td>
<td></td>
<td></td>
<td>0.53 (0.42 - 0.66)</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>288</td>
<td>147 (51)</td>
<td>260</td>
<td>78 (30)</td>
<td>1.00</td>
</tr>
<tr>
<td>Male</td>
<td>276</td>
<td>118 (43)</td>
<td>244</td>
<td>83 (34)</td>
<td>0.89 (0.69 - 1.16)</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;18</td>
<td>138</td>
<td>63 (46)</td>
<td>101</td>
<td>29 (29)</td>
<td>1.00</td>
</tr>
<tr>
<td>18-29</td>
<td>140</td>
<td>65 (46)</td>
<td>98</td>
<td>30 (31)</td>
<td>1.06 (0.72 - 1.56)</td>
</tr>
<tr>
<td>30-39</td>
<td>129</td>
<td>70 (54)</td>
<td>150</td>
<td>59 (39)</td>
<td>1.33 (0.93 - 1.92)</td>
</tr>
<tr>
<td>40-49</td>
<td>95</td>
<td>49 (52)</td>
<td>75</td>
<td>23 (31)</td>
<td>1.16 (0.78 - 1.73)</td>
</tr>
<tr>
<td>50+</td>
<td>62</td>
<td>18 (29)</td>
<td>80</td>
<td>20 (25)</td>
<td>0.58 (0.36 - 0.95)</td>
</tr>
<tr>
<td><strong>p-value, test for trend</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.209</td>
</tr>
<tr>
<td><strong>Occupation†</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not farmer</td>
<td>37</td>
<td>13 (35)</td>
<td>35</td>
<td>9 (26)</td>
<td>1.00</td>
</tr>
<tr>
<td>Farmer</td>
<td>389</td>
<td>189 (49)</td>
<td>368</td>
<td>123 (33)</td>
<td>1.59 (0.84 - 3.01)</td>
</tr>
<tr>
<td><strong>Education†</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elementary school or less</td>
<td>252</td>
<td>134 (53)</td>
<td>240</td>
<td>81 (34)</td>
<td>1.00</td>
</tr>
<tr>
<td>Some middle school or higher</td>
<td>173</td>
<td>68 (39)</td>
<td>162</td>
<td>51 (31)</td>
<td>0.70 (0.51 - 0.96)</td>
</tr>
<tr>
<td><strong>Village</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tuanjie 2</td>
<td>58</td>
<td>6 (10)</td>
<td>56</td>
<td>8 (14)</td>
<td>0.19 (0.09 - 0.39)</td>
</tr>
<tr>
<td>Jiaojia 4</td>
<td>59</td>
<td>13 (22)</td>
<td>54</td>
<td>7 (13)</td>
<td>0.29 (0.15 - 0.54)</td>
</tr>
<tr>
<td>Hexing 1</td>
<td>61</td>
<td>19 (31)</td>
<td>59</td>
<td>8 (14)</td>
<td>0.39 (0.22 - 0.71)</td>
</tr>
<tr>
<td>Minhe 1</td>
<td>67</td>
<td>29 (43)</td>
<td>55</td>
<td>23 (42)</td>
<td>0.99 (0.57 - 1.72)</td>
</tr>
<tr>
<td>Xinming 3</td>
<td>63</td>
<td>32 (51)</td>
<td>54</td>
<td>18 (33)</td>
<td>1.00</td>
</tr>
<tr>
<td>Minhe 3</td>
<td>61</td>
<td>35 (57)</td>
<td>56</td>
<td>20 (36)</td>
<td>1.18 (0.71 - 1.99)</td>
</tr>
<tr>
<td>Xinlong 7</td>
<td>45</td>
<td>27 (60)</td>
<td>38</td>
<td>16 (42)</td>
<td>1.45 (0.80 - 2.62)</td>
</tr>
<tr>
<td>Jianxing 6</td>
<td>60</td>
<td>41 (68)</td>
<td>48</td>
<td>21 (44)</td>
<td>1.81 (1.09 - 3.03)</td>
</tr>
<tr>
<td>Xinming 7</td>
<td>45</td>
<td>31 (69)</td>
<td>42</td>
<td>19 (45)</td>
<td>1.82 (1.06 - 3.11)</td>
</tr>
<tr>
<td>Shian 5</td>
<td>45</td>
<td>32 (71)</td>
<td>42</td>
<td>21 (50)</td>
<td>2.08 (1.14 - 3.81)</td>
</tr>
</tbody>
</table>

*Calculated by treating the age categories as ordinal.
†Includes adults (>18) only.
Odds ratios account for multiple observations from the same individual using GEE logistic regression with exchangeable correlation.

Half (51%) were female. Most adults (>18 years) were farmers (91%) and had no formal schooling beyond elementary school (59%). Ultrasound participants were slightly older than the rest of the population (mean age 29.8 vs. 28.2, p=0.037).

In 2002, 444 people had a second ultrasound examination and 321 people were examined using ultrasound in 2005. The 320 people with complete ultrasound follow-up were no more likely to be infected with *S. japonicum* at enrollment than those who missed at least one follow-up.
Table 3. Prevalence of abnormal liver and spleen measurements from 2000 to 2005 detected using ultrasound.

<table>
<thead>
<tr>
<th></th>
<th>2000</th>
<th>2002</th>
<th>2005</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%)</td>
<td>No. (%)</td>
<td>No. (%)</td>
</tr>
<tr>
<td>Parenchymal fibrosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>388 (67)</td>
<td>297 (67)</td>
<td>219 (68)</td>
</tr>
<tr>
<td>Grade 1</td>
<td>83 (14)</td>
<td>50 (11)</td>
<td>35 (11)</td>
</tr>
<tr>
<td>Grade 2</td>
<td>92 (16)</td>
<td>88 (20)</td>
<td>58 (18)</td>
</tr>
<tr>
<td>Grade 3</td>
<td>15 (3)</td>
<td>9 (2)</td>
<td>9 (3)</td>
</tr>
<tr>
<td>Periportal fibrosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 0 (av. diameter &lt;3 mm)</td>
<td>22 (4)</td>
<td>24 (5)</td>
<td>19 (6)</td>
</tr>
<tr>
<td>Grade 1 (av. diameter 3 – 5 mm)</td>
<td>535 (93)</td>
<td>401 (90)</td>
<td>299 (93)</td>
</tr>
<tr>
<td>Grade 2 (av. diameter 5.1 – 7 mm)</td>
<td>21 (4)</td>
<td>19 (4)</td>
<td>3 (1)</td>
</tr>
<tr>
<td>Grade 3 (av. diameter &gt;7mm)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Left liver lobe*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>389 (84)</td>
<td>373 (85)</td>
<td>250 (79)</td>
</tr>
<tr>
<td>Enlarged</td>
<td>73 (16)</td>
<td>67 (15)</td>
<td>67 (21)</td>
</tr>
<tr>
<td>Right liver lobe*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>351 (76)</td>
<td>402 (91)</td>
<td>277 (87)</td>
</tr>
<tr>
<td>Enlarged</td>
<td>111 (24)</td>
<td>38 (9)</td>
<td>40 (13)</td>
</tr>
<tr>
<td>Portal vein*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>417 (90)</td>
<td>383 (87)</td>
<td>274 (86)</td>
</tr>
<tr>
<td>Dilated</td>
<td>45 (10)</td>
<td>57 (13)</td>
<td>43 (14)</td>
</tr>
<tr>
<td>Spleen (thickness)*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>442 (96)</td>
<td>425 (97)</td>
<td>310 (98)</td>
</tr>
<tr>
<td>Enlarged</td>
<td>18 (4)</td>
<td>14 (3)</td>
<td>6 (2)</td>
</tr>
<tr>
<td>Spleen vein†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>549 (95)</td>
<td>406 (92)</td>
<td>313 (98)</td>
</tr>
<tr>
<td>Dilated</td>
<td>28 (5)</td>
<td>37 (8)</td>
<td>7 (2)</td>
</tr>
</tbody>
</table>

*Portal vein dilation and enlargement of the left liver lobe, right liver lobe and spleen were classified based on height-specific normal values [18].
†The spleen vein was classified as dilated if the diameter exceeded 8 mm.

up examination. But people with three ultrasound examinations were older (mean age 33.0 vs. 25.6, p<0.001) and more likely to have at least one liver abnormality in 2000 (57% vs. 28%, p<0.001) that those with incomplete follow-up. Nearly half (47%) of participants tested positive for S. japonicum in 2000. Mean infection intensity was 53.4 EPG. In 2002, infection prevalence declined to 32% and intensity to 9.4 EPG (Table 2). There was no difference in infection prevalence by sex or occupation. Adults aged 50 and older were less likely to be infected than younger participants. Adults who had attended middle school or higher were less likely to be infected than adults with less formal schooling. People infected in 2000 were almost twice as likely to be infected again in 2002 compared to individuals who were not infected at baseline, despite prompt praziquantel treatment of everyone who tested positive (41% vs. 23%, p<0.001). Infection prevalence varied significantly by village.
Table 4. Unadjusted odds ratios showing the distribution of ultrasound-detected abnormalities of the liver by age and sex.

<table>
<thead>
<tr>
<th>Age</th>
<th>Parenchymal fibrosis OR (95%CI)</th>
<th>Periportal fibrosis OR (95%CI)</th>
<th>Left liver lobe enlargement OR (95%CI)</th>
<th>Right liver lobe enlargement OR (95%CI)</th>
<th>Portal vein dilation OR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Male</td>
<td>1.18 (0.85 - 1.64)</td>
<td>4.35 (2.05 - 9.23)</td>
<td>0.41 (0.28 - 0.60)</td>
<td>1.88 (1.29 - 2.73)</td>
<td>1.28 (0.87 - 1.89)</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 18</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>18 - 29</td>
<td>10.44 (5.11 - 21.33)</td>
<td>9.36 (1.14 - 76.56)</td>
<td>2.91 (1.35 - 6.29)</td>
<td>2.40 (1.25 - 4.61)</td>
<td>2.00 (0.93 - 4.33)</td>
</tr>
<tr>
<td>30 - 39</td>
<td>11.76 (5.96 - 23.18)</td>
<td>9.79 (1.31 - 73.01)</td>
<td>4.00 (2.03 - 7.89)</td>
<td>2.91 (1.61 - 5.28)</td>
<td>3.19 (1.58 - 6.44)</td>
</tr>
<tr>
<td>40 - 49</td>
<td>30.76 (15.21 - 62.20)</td>
<td>10.95 (1.41 - 85.30)</td>
<td>4.56 (2.25 - 9.23)</td>
<td>3.13 (1.65 - 5.95)</td>
<td>2.82 (1.35 - 5.91)</td>
</tr>
<tr>
<td>≥ 50</td>
<td>35.89 (17.45 - 73.80)</td>
<td>8.47 (1.07 - 67.07)</td>
<td>6.21 (3.05 - 12.63)</td>
<td>3.26 (1.66 - 6.41)</td>
<td>3.98 (1.97 - 8.06)</td>
</tr>
<tr>
<td><strong>trend†</strong></td>
<td>&lt;0.001</td>
<td>0.025</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*There were no children (<18) with grade 3 parenchymal fibrosis, leading to a violation of the parallel regression assumption for ordinal logistic regression. Grade 3 was grouped with grade 2 in order to avoid a model violation.
†Test for trend was calculated treating the age categories as ordinal. P-values reported.

Odds ratios account for multiple observations from the same individual. Ordinal logistic regression with a sandwich type estimator was used for parenchymal fibrosis. GEE logistic regression with exchangeable correlation was used for all other measures.
Table 5. Unadjusted odds ratios showing the distribution of ultrasound-detected abnormalities of the spleen by age and sex.

<table>
<thead>
<tr>
<th></th>
<th>Spleen enlargement OR (95%CI)</th>
<th>Spleen vein dilation OR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Male</td>
<td>0.89 (0.43 - 1.83)</td>
<td>1.22 (0.74 - 2.01)</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 18</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>18 - 29</td>
<td>3.40 (1.02 - 11.34)</td>
<td>3.18 (1.34 - 7.58)</td>
</tr>
<tr>
<td>30 - 39</td>
<td>2.09 (0.63 - 6.88)</td>
<td>2.60 (1.11 - 6.12)</td>
</tr>
<tr>
<td>40 - 49</td>
<td>1.74 (0.45 - 6.78)</td>
<td>1.35 (0.46 - 3.94)</td>
</tr>
<tr>
<td>≥ 50</td>
<td>1.82 (0.47 - 6.98)</td>
<td>2.32 (0.93 - 5.79)</td>
</tr>
<tr>
<td><strong>trend</strong></td>
<td>0.894</td>
<td>0.548</td>
</tr>
</tbody>
</table>

*Test for trend was calculated treating the age categories as ordinal. P-values reported.
Odds ratios account for multiple observations from the same individual using generalized estimating equations logistic regression with exchangeable correlation.

At baseline, 19% of participants had grade 2 or 3 parenchymal fibrosis and this unadjusted prevalence changed little over the five year follow-up period (Table 3). Periportal fibrosis was less common than parenchymal fibrosis. Grade 2 periportal fibrosis was detected in 4% of participants at baseline and 1% of participants at five-year follow-up. No grade 3 periportal fibrosis was detected throughout the study. Spleen enlargement and dilation of the spleen vein were also rare, detected in 4% and 5% of participants at baseline, respectively. Left and right liver lobe enlargement were more prevalent, detected in 16% and 24% of participants at baseline and 21% and 13% of participants at five-year follow-up.

Liver abnormalities increased significantly with age, most notably for parenchymal fibrosis (Table 4). Spleen enlargement and spleen vein dilation were most prevalent in young adults aged 18 to 29 years (Table 5). The relationship between liver abnormalities and sex varied: men were more likely to have periportal fibrosis and enlarged right liver lobes; women were more likely to have enlarged left liver lobes. Spleen enlargement and vein dilation did not vary between males and females.

Following the initiation of schistosomiasis testing and treatment of all infections in 2000, the prevalence of parenchymal fibrosis, periportal fibrosis and right liver lobe enlargement decreased significantly through 2005, controlling for age and sex (Table 6). Decreases in spleen enlargement were also observed, although the trend was of marginal significance (p=0.091). Left liver lobe enlargement, portal vein dilation and spleen vein diameter did not decrease significantly after the initiation of treatment.

Schistosomiasis infection at the time of ultrasound was associated with an increase in liver parenchymal fibrosis grade (OR 1.40, 95% CI: 1.03 – 1.90), adjusting for age, sex and year of ultrasound (Table 7). Infection intensity at the time of ultrasound was also associated with an increase in liver parenchymal fibrosis grade (test for trend: p=0.002). Individuals with greater than 50 EPG had 2.10 times greater odds of more advanced fibrosis than those not excreting eggs (95%CI: 1.33 - 3.32). Schistosomiasis infection two to three years prior to ultrasound was associated with an increase in liver parenchymal fibrosis grade, and the association was stronger...
Table 6. Changes in ultrasound-detected morbidity two and five years after the initiation of *S. japonicum* infection testing and treatment compared to baseline (2000).

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted OR (95%CI)</th>
<th>Adjusted* OR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parenchymal fibrosis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>2002</td>
<td>1.05 (0.90 - 1.22)</td>
<td>0.82 (0.68 - 0.98)</td>
</tr>
<tr>
<td>2005</td>
<td>0.99 (0.81 - 1.22)</td>
<td>0.57 (0.44 - 0.73)</td>
</tr>
<tr>
<td>p-value, test for trend†</td>
<td>0.976</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Periportal fibrosis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>2002</td>
<td>1.16 (0.64 - 2.08)</td>
<td>1.07 (0.59 - 1.96)</td>
</tr>
<tr>
<td>2005</td>
<td>0.22 (0.06 - 0.82)</td>
<td>0.20 (0.06 - 0.63)</td>
</tr>
<tr>
<td>p-value, test for trend†</td>
<td>0.012</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>Left liver lobe enlargement</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>2002</td>
<td>0.94 (0.69 - 1.28)</td>
<td>0.86 (0.62 - 1.19)</td>
</tr>
<tr>
<td>2005</td>
<td>1.36 (0.97 - 1.91)</td>
<td>1.11 (0.77 - 1.60)</td>
</tr>
<tr>
<td>p-value, test for trend†</td>
<td>0.104</td>
<td>0.634</td>
</tr>
<tr>
<td><strong>Right liver lobe enlargement</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>2002</td>
<td>0.29 (0.21 - 0.41)</td>
<td>0.26 (0.18 - 0.37)</td>
</tr>
<tr>
<td>2005</td>
<td>0.45 (0.31 - 0.64)</td>
<td>0.35 (0.24 - 0.52)</td>
</tr>
<tr>
<td>p-value, test for trend†</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Portal vein dilation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>2002</td>
<td>1.37 (0.94 - 2.00)</td>
<td>1.28 (0.87 - 1.88)</td>
</tr>
<tr>
<td>2005</td>
<td>1.44 (0.94 - 2.19)</td>
<td>1.20 (0.78 - 1.84)</td>
</tr>
<tr>
<td>p-value, test for trend†</td>
<td>0.074</td>
<td>0.362</td>
</tr>
<tr>
<td><strong>Spleen enlargement</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>2002</td>
<td>0.80 (0.43 - 1.51)</td>
<td>0.79 (0.42 - 1.49)</td>
</tr>
<tr>
<td>2005</td>
<td>0.45 (0.17 - 1.22)</td>
<td>0.43 (0.16 - 1.20)</td>
</tr>
<tr>
<td>p-value, test for trend†</td>
<td>0.102</td>
<td>0.091</td>
</tr>
<tr>
<td><strong>Spleen vein dilation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>2002</td>
<td>1.85 (1.13 - 3.03)</td>
<td>1.79 (1.09 - 2.93)</td>
</tr>
<tr>
<td>2005</td>
<td>0.44 (0.19 - 1.03)</td>
<td>0.41 (0.17 - 0.95)</td>
</tr>
<tr>
<td>p-value, test for trend†</td>
<td>0.195</td>
<td>0.119</td>
</tr>
</tbody>
</table>

*Adjusted for age and sex.
†Calculated treating the categories as ordinal.
Odds ratios account for multiple observations from the same individual. Ordinal logistic regression with a sandwich type estimator was used for parenchymal fibrosis. GEE logistic regression with exchangeable correlation was used for all other measures.
Table 7. The associations between of current and prior *S. japonicum* infection and liver fibrosis. Current infection was measured at the time of the ultrasound examination. Prior infection was measured two to three years prior to the ultrasound examination.

<table>
<thead>
<tr>
<th></th>
<th>Parenchymal fibrosis</th>
<th>Periportal fibrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unadjusted OR (95% CI)</td>
<td>Adjusted* OR (95% CI)</td>
</tr>
<tr>
<td>I. Current infection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uninfected</td>
<td>1.00 (1.00)</td>
<td>1.00</td>
</tr>
<tr>
<td>Infected</td>
<td>1.21 (0.91 - 1.61)</td>
<td>1.40 (1.03 - 1.90)</td>
</tr>
<tr>
<td>II. Current infection intensity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 EPG</td>
<td>1.00 (1.00)</td>
<td>1.00</td>
</tr>
<tr>
<td>1 - 10 EPG</td>
<td>1.36 (0.91 - 2.05)</td>
<td>1.50 (0.99 - 2.27)</td>
</tr>
<tr>
<td>11 - 50 EPG</td>
<td>1.22 (0.81 - 1.85)</td>
<td>1.30 (0.82 - 2.08)</td>
</tr>
<tr>
<td>&gt;50 EPG</td>
<td>1.46 (0.95 - 2.25)</td>
<td>2.10 (1.33 - 3.32)</td>
</tr>
<tr>
<td>p-value, test for trend†</td>
<td></td>
<td>0.052</td>
</tr>
<tr>
<td>III. Prior infection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uninfected</td>
<td>1.00 (1.00)</td>
<td>1.00</td>
</tr>
<tr>
<td>Infected</td>
<td>1.48 (1.06 - 2.05)</td>
<td>1.84 (1.30 - 2.60)</td>
</tr>
<tr>
<td>IV. Prior infection intensity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 EPG</td>
<td>1.00 (1.00)</td>
<td>1.00</td>
</tr>
<tr>
<td>1 - 10 EPG</td>
<td>1.42 (0.90 - 2.24)</td>
<td>1.71 (1.04 - 2.79)</td>
</tr>
<tr>
<td>11 - 50 EPG</td>
<td>1.79 (1.08 - 2.97)</td>
<td>2.07 (1.21 - 3.57)</td>
</tr>
<tr>
<td>&gt;50 EPG</td>
<td>1.81 (1.14 - 2.89)</td>
<td>2.84 (1.71 - 4.73)</td>
</tr>
<tr>
<td>p-value, test for trend†</td>
<td></td>
<td>0.002</td>
</tr>
</tbody>
</table>

*Adjusted for age, sex and year of ultrasound.
†Calculated treating the categories as ordinal.
‡Models did not yield stable estimates, likely due to the low prevalence of periportal fibrosis.

Odds ratios account for multiple observations from the same individual. Ordinal logistic regression with a sandwich type estimator was used for parenchymal fibrosis. GEE logistic regression with exchangeable correlation was used for periportal fibrosis.

than that of current infection (OR 1.84, 95% CI: 1.30 – 2.60). Prior infection intensity was also associated with liver parenchymal fibrosis (test for trend, p<0.001). Individuals with greater than 50 EPG two to three years prior to the ultrasound examination had 2.84 times greater odds of advanced fibrosis than those not excreting eggs two to three years prior to ultrasound (95% CI: 1.71 - 4.73).

The other hepatosplenic ultrasound measures, including periportal fibrosis, left and right liver lobe enlargement, portal vein dilation, spleen enlargement and spleen vein dilation, were not associated with current infection status or intensity. Several measures were associated with prior infection, although the relationships were not as consistent as those observed for parenchymal fibrosis. Prior infection but not prior infection intensity appeared to elevate the probability of left liver lobe enlargement (OR 1.45, 95% CI: 0.97 – 2.17) (Table 8). In contrast, prior infection intensity but not prior infection status was associated with increased odds of portal vein dilation (test for trend, p=0.051). The impact of prior infection status on right liver lobe enlargement varied by the year of examination and the sex of the participant (Table 9).
### Table 8

The associations between current and prior *S. japonicum* infection and left liver lobe enlargement and portal vein dilation. Current infection was measured at the time of the ultrasound examination. Prior infection was measured two to three years prior to the ultrasound examination.

<table>
<thead>
<tr>
<th></th>
<th>Left liver lobe enlargement</th>
<th>Portal vein dilation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unadjusted</td>
<td>Adjusted*</td>
</tr>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>I. Current infection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uninfected</td>
<td>1.00 (1.00)</td>
<td>1.00</td>
</tr>
<tr>
<td>Infected</td>
<td>1.00 (0.72 - 1.40)</td>
<td>1.05 (0.73 - 1.51)</td>
</tr>
<tr>
<td>II. Current infection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>intensity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 EPG</td>
<td>1.00 (1.00)</td>
<td>1.00</td>
</tr>
<tr>
<td>1 - 10 EPG</td>
<td>1.02 (0.55 - 1.91)</td>
<td>1.04 (0.55 - 1.97)</td>
</tr>
<tr>
<td>11 - 50 EPG</td>
<td>0.93 (0.51 - 1.69)</td>
<td>0.94 (0.50 - 1.75)</td>
</tr>
<tr>
<td>&gt;50 EPG</td>
<td>1.47 (0.84 - 2.55)</td>
<td>1.71 (0.94 - 3.12)</td>
</tr>
<tr>
<td>p-value, test for trend†</td>
<td>0.301</td>
<td>0.185</td>
</tr>
<tr>
<td>III. Prior infection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uninfected</td>
<td>1.00 (1.00)</td>
<td>1.00</td>
</tr>
<tr>
<td>Infected</td>
<td>1.18 (0.81 - 1.72)</td>
<td>1.45 (0.97 - 2.17)</td>
</tr>
<tr>
<td>IV. Prior infection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>intensity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 EPG</td>
<td>1.00 (1.00)</td>
<td>1.00</td>
</tr>
<tr>
<td>1 - 10 EPG</td>
<td>1.07 (0.62 - 1.86)</td>
<td>1.21 (0.68 - 2.15)</td>
</tr>
<tr>
<td>11 - 50 EPG</td>
<td>1.17 (0.67 - 2.04)</td>
<td>1.39 (0.79 - 2.42)</td>
</tr>
<tr>
<td>&gt;50 EPG</td>
<td>0.79 (0.41 - 1.52)</td>
<td>1.12 (0.57 - 2.20)</td>
</tr>
<tr>
<td>p-value, test for trend†</td>
<td>0.712</td>
<td>0.440</td>
</tr>
</tbody>
</table>

*Adjusted for age, sex and year of ultrasound.
†Calculated treating the categories as ordinal.

Odds ratios account for multiple observations from the same individual using GEE logistic regression with exchangeable correlation.

Prior infection was associated with increased odds of right liver lobe enlargement among males examined in 2005 (OR 3.95, 95%CI: 1.82 – 8.57) and decreased odds of right liver lobe enlargement among females examined in 2002 (OR 0.33, 95% CI: 0.13 – 0.86). The odds of periportal fibrosis were higher in people who were currently infected with *S. japonicum* and highest in those with greater than 50 EPG but this relationship was not statistically significant. Due to the limited number of individuals with periportal fibrosis, models were unable to yield stable estimates of the effect of prior infection on this measure. Spleen enlargement and spleen vein dilation were not associated with current or prior infection (Table 10).

Most people with liver and spleen enlargement, portal vein dilation, periportal fibrosis or spleen vein dilation in 2000 had normal pathology by 2002 (Table 11). However, this was not the case for parenchymal fibrosis: 67% of people with grade 2 fibrosis and 100% with grade 3 fibrosis in 2000 remained at or above grade 2 throughout the five-year follow-up.
Table 9. The associations between current and prior *S. japonicum* infection and right liver lobe enlargement. Current infection was measured at the time of the ultrasound examination. Prior infection was measured two to three years prior to the ultrasound examination.

<table>
<thead>
<tr>
<th></th>
<th>Right liver lobe enlargement</th>
<th>Unadjusted</th>
<th>OR (95% CI)</th>
<th>Adjusted*</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Current infection</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uninfected</td>
<td>1.00</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infected</td>
<td>1.04 (0.72 - 1.49)</td>
<td>0.92 (0.63 - 1.35)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II. Current infection intensity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 EPG</td>
<td>1.00</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 - 10 EPG</td>
<td>0.54 (0.27 - 1.11)</td>
<td>0.50 (0.24 - 1.03)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11 - 50 EPG</td>
<td>1.22 (0.68 - 2.21)</td>
<td>0.96 (0.54 - 1.72)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;50 EPG</td>
<td>1.77 (1.06 - 2.98)</td>
<td>1.37 (0.77 - 2.44)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-value, test for trend†</td>
<td>0.067</td>
<td>0.499</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III. Prior infection‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females in 2002</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uninfected</td>
<td>1.00</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infected</td>
<td>0.33 (0.13 - 0.86)</td>
<td>0.93 (0.38 - 2.32)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females in 2005</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uninfected</td>
<td>1.00</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infected</td>
<td>1.40 (0.65 – 3.00)</td>
<td>3.95 (1.82 - 8.57)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males in 2002</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uninfected</td>
<td>1.00</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infected</td>
<td>3.95 (1.82 - 8.57)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Adjusted for age, sex and year of ultrasound.
†Calculated treating the categories as ordinal.
‡Interactions between prior infection status and sex, and prior infection status and year of ultrasound were detected (p=0.005 and p=0.031, respectively)
Odds ratios account for multiple observations from the same individual using GEE logistic regression with exchangeable correlation.

Discussion

We found evidence of a direct, exposure-response relationship between *S. japonicum* infection and parenchymal fibrosis. While there has been suggestive evidence of an association between schistosomiasis and parenchymal fibrosis, including an association between progression of parenchymal fibrosis and current infection [16], this is the first study to show the risk of parenchymal fibrosis is higher in people who are infected, and highest in individuals with the greatest infection intensities. Parenchymal fibrosis declined significantly following treatment, but improvements were limited among individuals with advanced fibrosis: 72% of people with severe fibrosis at enrollment (grades 2 or 3) had not resolved below grade 2 by the end of the
Table 10. The associations between current and prior *S. japonicum* infection and ultrasound-detected spleen abnormalities. Current infection was measured at the time of the ultrasound examination. Prior infection was measured two to three years prior to the ultrasound examination.

<table>
<thead>
<tr>
<th></th>
<th>Spleen enlargement</th>
<th>Spleen vein dilation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unadjusted OR (95% CI)</td>
<td>Adjusted* OR (95% CI)</td>
</tr>
<tr>
<td>I. Current infection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uninfected</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Infected</td>
<td>0.96 (0.47 - 1.95)</td>
<td>0.92 (0.44 - 1.94)</td>
</tr>
<tr>
<td>II. Current infection intensity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 EPG</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>1 - 10 EPG</td>
<td>1.44 (0.56 - 3.66)</td>
<td>1.38 (0.54 - 3.51)</td>
</tr>
<tr>
<td>11 - 50 EPG</td>
<td>0.72 (0.18 - 2.82)</td>
<td>0.64 (0.15 - 2.72)</td>
</tr>
<tr>
<td>&gt;50 EPG</td>
<td>0.50 (0.13 - 1.99)</td>
<td>0.46 (0.11 - 1.86)</td>
</tr>
<tr>
<td>p-value, test for trend†</td>
<td>0.360</td>
<td>0.280</td>
</tr>
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<td>III. Prior infection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uninfected</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Infected</td>
<td>1.16 (0.47 - 2.83)</td>
<td>1.00 (0.41 - 2.44)</td>
</tr>
<tr>
<td>IV. Prior infection intensity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 EPG</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>1 - 10 EPG</td>
<td>0.61 (0.13 - 2.89)</td>
<td>0.59 (0.11 - 3.21)</td>
</tr>
<tr>
<td>11 - 50 EPG</td>
<td>2.34 (0.72 - 7.66)</td>
<td>1.88 (0.56 - 6.37)</td>
</tr>
<tr>
<td>&gt;50 EPG</td>
<td>1.09 (0.23 - 5.14)</td>
<td>0.82 (0.16 - 4.32)</td>
</tr>
<tr>
<td>p-value, test for trend†</td>
<td>0.500</td>
<td>0.835</td>
</tr>
</tbody>
</table>

*Adjusted for age, sex and year of ultrasound.
†Calculated treating the categories as ordinal.

Odds ratios account for multiple observations from the same individual using GEE logistic regression with exchangeable correlation.

five-year study. This suggests parenchymal fibrosis is an appropriate measure of *S. japonicum* morbidity and can be used document improvements in morbidity following treatment, although little improvement may be observed among those with advanced fibrosis.

In contrast, the remaining measures were less consistently associated with *S. japonicum* infection and are of questionable epidemiological use in regions with similar infection levels. Periportal fibrosis was rare in this population and could not be associated with *S. japonicum* infection, although it did decline significantly following the initiation of targeted treatment. Others have used image-based classification to assess periportal fibrosis [34,35], which has been shown to have better reproducibility for *S. mansoni*-related fibrosis, perhaps because the method does not require the ultrasonographer to measure narrow vessel widths [36]. The measures of periportal fibrosis used here were not height adjusted, as Chinese standards for the diameter of portal vein branches as measured here have not been published. The lack of height adjustment may have led to underestimates of the prevalence of periportal fibrosis and may explain the higher prevalence of periportal fibrosis in men. But higher prevalence of fibrosis has also been observed in males using image-based classification [35]. The use of a qualitative measure of periportal fibrosis to assess *S. japonicum* morbidity warrants consideration.
Table 11. The reversal of ultrasound-detected morbidity among individuals with abnormal measures in 2000.

<table>
<thead>
<tr>
<th>Measure</th>
<th>No. in 2000*</th>
<th>Reversed in 2002 No. (%)</th>
<th>Reversed in 2005 No. (%)</th>
<th>Never reversed No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parenchymal fibrosis (grade 2 or 3)†</td>
<td>71</td>
<td>11 (15)</td>
<td>17 (24)</td>
<td>51 (72)</td>
</tr>
<tr>
<td>Periportal fibrosis (grade 2)</td>
<td>17</td>
<td>14 (82)</td>
<td>16 (94)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Left liver lobe enlarged</td>
<td>58</td>
<td>35 (60)</td>
<td>35 (60)</td>
<td>13 (22)</td>
</tr>
<tr>
<td>Right liver lobe enlarged</td>
<td>85</td>
<td>65 (76)</td>
<td>63 (74)</td>
<td>10 (12)</td>
</tr>
<tr>
<td>Portal vein dilated</td>
<td>33</td>
<td>22 (67)</td>
<td>24 (73)</td>
<td>5 (15)</td>
</tr>
<tr>
<td>Spleen enlarged</td>
<td>16</td>
<td>13 (81)</td>
<td>16 (100)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Spleen vein dilated</td>
<td>16</td>
<td>12 (75)</td>
<td>15 (94)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

*Includes only individuals with complete follow-up data.
†Reversal of parenchymal fibrosis occurred when participants were assigned grades 0 or 1 at follow-up.

The remaining three hepatic measures, left and right liver lobe enlargement and portal vein dilation, were associated with *S. japonicum* infection or declined following chemotherapy. However, the relationships between *S. japonicum* infection and these morbidity measures were less consistent than those observed with parenchymal fibrosis, and several associations were of marginal statistical significance. The spleen measures were neither associated with infection nor did they decline with treatment.

The prevalence of morbidity in this cohort was lower than has been observed elsewhere in China, as was the infection intensity [16,17,34,37]. Measures of liver and spleen enlargement, spleen vein dilation and portal vein dilation may be appropriate in areas where infection intensity and associated morbidities are higher. Declines in hepatomegaly and splenomegaly have been demonstrated following treatment in regions with higher average worm burdens [16,17].

The strong associations between age and all liver measures highlight the importance of considering age as a potential confounder when examining the relationship between *S. japonicum* infection and morbidity or declines in morbidity over time. This is perhaps most notable in the adjusted vs. unadjusted estimates of changes of parenchymal fibrosis from 2000 to 2005. The unadjusted odds ratios were close to 1.00, indicating no decline in fibrosis following the initiation of *S. japonicum* testing and treatment. However loss to follow-up was highest in younger populations, which were least likely to have fibrosis. When age and sex were included in the models, the risk of parenchymal fibrosis was reduced by approximately 18% in 2002 and 43% in 2005.

Community-wide testing and treatment of all infections with praziquantel yielded marked declines in infection prevalence and intensity. However, reinfection was high: 32% of people were infected two years after the first round of targeted treatment. High rates of reinfection following treatment are not uncommon and underscore the challenges of sustainably reducing human schistosomiasis [38]. Adults age 50 and over were less likely to be infected than younger populations, possibly corresponding to a decline in water contact later in life or acquired immunity [39].

The findings presented here provide insights into the development and repair of hepatic fibrosis following *S. japonicum* infection. The lack of reversal of grade 3 parenchymal fibrosis (to grade 1 or normal) suggests severe fibrosis may persist despite treatment. Prior studies have also found minimal reversal of severe fibrosis following treatment [16,40] and hepatic fibrosis
has been documented in people living in areas where the parasite, and therefore exposure, was eliminated 20 years previously [41]. While treatment with praziquantel can reduce infection prevalence, intensity and fibrosis, this analysis provides further evidence that severe hepatic fibrosis is unlikely to reverse quickly if at all. Minimal declines in severe hepatic fibrosis associated with *S. mansoni* infection have also been detected [42] but other studies have noted complete reversal of severe morbidity [43].

The association between past infection and fibrosis raises questions about the progression of fibrosis following infection and treatment. Parenchymal fibrosis was associated with current infection status and intensity but more strongly associated with infection status and intensity two to three years prior to ultrasound, despite timely treatment of infections. Praziquantel kills the adult worm but schistosome eggs can remain trapped in host tissues decades after exposure [44]. While schistosome eggs are thought to disintegrate within weeks of granuloma formation [7], it is possible, due to an inflammatory cascade, for fibrogenesis to continue over a longer time period. Collagen deposition has been shown to occur following treatment with praziquantel in *S. japonicum*-infected mice, suggesting fibrosis may continue despite removal of adult worms [45]. In humans, progression of fibrosis has been observed following treatment and was not explained by reinfection [21,46]. Alternatively, the association between past infection and fibrosis may be due to reinfection as past infection predicts subsequent infection. However, if reinfection, rather than past infection, determines fibrosis, one would expect stronger relationships between current infection and fibrosis than between past infection and fibrosis, which were not observed. It is also possible that some individuals who were treated were not cured. A single dose of praziquantel cures 90% of *S. japonicum* infections [47], suggesting 10% of those treated may continue to harbor adult worms and face continued egg production. In my work in Sichuan, I have also found individuals who decline to take praziquantel even after a positive infection test. Non-compliance and treatment failure are realities of any chemotherapy-based control program, but the marked declines in infection prevalence, intensity and morbidity suggest the number of individuals who were not cured was limited.

This study examined one of the largest populations to be followed over five years in order to assess ultrasound-detected morbidity and *S. japonicum* infection. Participants were randomly sampled from a comprehensive cross-sectional survey in order to minimize selection bias. Loss to follow-up was greater among those without ultrasound-detected morbidity at baseline, which suggests the prevalence of morbidity in 2002 and 2005 may be overestimated, and therefore the true declines in morbidity over time may be greater than observed. Retention was independent of infection status, minimizing bias in estimates of the impact of infection on pathology. No information was available on alcohol consumption or infection with hepatitis B virus (HBV), two factors that can induce liver pathology and may exacerbate schistosomiasis morbidity. The parenchymal network pattern due to *S. japonicum* is distinct from the lesions produced by HBV, as HBV produces a finer, meshwork texture [48], suggesting the observed prevalence of parenchymal fibrosis is specific to schistosomiasis. While HBV has been shown to hinder regression of periportal fibrosis following treatment of *S. mansoni* infections [42], the extent to which HBV exacerbates morbidity due to *S. japonicum* remains unclear and warrants further study. Reductions in parenchymal fibrosis may be impaired by alcohol consumption, which is confined almost exclusively to males in this study area [17]. Unless alcohol consumption or HBV impact a person’s probability of infection, they are unlikely to confound the relationships between infection and ultrasound-detected morbidity.
In summary, this study provides evidence that ultrasound-detected liver fibrosis is associated with *S. japonicum* infection status and intensity, controlling for age, sex and year of ultrasound examination, and this measure can be used to monitor *S. japonicum*-induced morbidity. Other ultrasound measures including hepatomegaly and splenomegaly were less clearly associated with infection and may be of limited epidemiological use in regions with similar infection intensities. These findings also suggest some morbidity may not reverse within five years of treatment and may even progress despite treatment. Praziquantel has yielded remarkable declines in schistosomiasis morbidity in China and throughout the globe but reinfection following treatment is common, as observed in this study population. *S. japonicum* infection, particularly high egg loads, may lead to fibrosis that is not rapidly reversed by treatment, underscoring the importance of measures to prevent new infections, as well as treating current disease.

References


CHAPTER 3

The challenges of surveillance following successful disease control: a survey of human, bovine and snail *Schistosoma japonicum* infections in a region where schistosomiasis reemerged

Introduction

China has achieved remarkable progress in controlling schistosomiasis. Transmission has been eliminated in five provinces and the number of infections reduced from approximately ten million in the 1950s to an estimated 726,000 in the most recent national survey [1,2]. Like many parasitic infections, schistosomiasis can be treated with a safe and inexpensive drug, praziquantel, but no vaccine is currently available and treatment does not protect against future infection. As China aims to control schistosomiasis transmission nationwide by 2015 [3], potentially providing a model for the elimination of schistosomiasis and other parasitic infections, the threat of reemergence looms large.

Limited outbreaks of schistosomiasis have been documented previously in China. An outbreak of schistosomiasis occurred at an urban factory in 1989 and was attributed to migrant workers [4], as was a small outbreak in Guangdong province over a decade ago [5]. The devastating 1998 flood in the Yangtze River Valley allowed the intermediate snail host to repopulate areas where it had been eradicated and to colonize previously non-endemic areas, leading to the emergence of human schistosomiasis [6].

Recent evidence suggests that schistosomiasis is reemerging at a scale that has not been observed previously. The Chinese Ministry of Health defines several benchmarks for the control of schistosomiasis: *transmission control* requires that human and cattle infection prevalence are less than 1% and no acute human schistosomiasis cases or incident infections in children (<12 years old) or young cattle (<2 years old) are detected; *transmission interruption* requires that human and bovine infection prevalence are less than 0.2%, incident bovine and human infections have not been detected for five consecutive years and the intermediate snail host has not been found for at least one year [7]. For the purpose of this paper, reemergence is defined as the return of human schistosomiasis in regions that have met transmission control or interruption benchmarks. In Sichuan province, reemergence has occurred in eight of the 46 counties where transmission was previously controlled [7]. Nationwide, 38 counties in the seven remaining endemic provinces have been classified as reemerging due to the detection of human infection prevalence above 1% [2,8].

Little is known about the epidemiology of reemerging schistosomiasis, including who is at greatest risk of infection. This lack of information creates a particular challenge for the design of post-control surveillance programs. To date, much of what is known about the reemergence
of schistosomiasis is based on the detection of cases of acute schistosomiasis and national infection surveys. Acute schistosomiasis, also called Katayama fever, is characterized by rapid and severe onset of fever, myalgia and eosinophilia that occurs shortly after infection [9]. Acute schistosomiasis from *S. mansoni* and *S. haematobium* is only seen in naïve populations, but *S. japonicum* can trigger acute responses in people who have been infected previously [9,10]. In China, acute schistosomiasis presents most frequently in children and is a reportable disease [11]. While acute infections clearly signal the return of human schistosomiasis to a region, it is possible schistosomiasis might return more quietly, in the form of chronic infections. It is unknown how sensitive acute case monitoring is in the detection of schistosomiasis reemergence. National infection surveys can provide estimates of human infections throughout endemic and controlled regions, but due to their cost, they occur approximately once every five to ten years and are impractical for short-term surveillance. In the five provinces where schistosomiasis transmission control has been attained, surveillance for reemergence is centered around monitoring the snail host *Oncomelania hupensis*, including surveys to assess snail densities and snail *S. japonicum* infections [5]. While infected snails are essential to schistosomiasis transmission, infected snail densities can be low and the sensitivities of snail-based surveillance methods are unknown. The long-term control of schistosomiasis hinges on the ability to detect the return of the parasite in a timely manner so that control measures can be implemented and widespread reemergence avoided.

This study was designed to provide one of the first in-depth examinations of schistosomiasis transmission in a region where reemergence has occurred, and to evaluate post-control surveillance strategies. Specifically, I set out to document the distribution of *S. japonicum* infections in human, snail and bovine populations in three counties where schistosomiasis reemerged following the declaration of transmission control. In addition, I aimed to assess the performance of surveillance techniques, including surveys for *S. japonicum*-infected snails, acute schistosomiasis case reporting and targeted host population surveys, testing the hypothesis that acute case surveillance and infected snail surveys have low sensitivities in detecting the presence of human schistosomiasis relative to targeted monitoring of mammalian populations.

**Methods**

Continuing the work of Liang et al. [7], three of the eight counties where schistosomiasis reemergence was detected in Sichuan province following transmission control were selected for inclusion in this study. Counties were selected based on the availability of surveillance records and the willingness of the control station personnel to collaborate on this project. Due to the sensitive nature of conducting infection surveys in regions where schistosomiasis transmission control goals have officially been met and to promote candid reporting, the names and exact locations of the counties and study villages have been withheld.

County schistosomiasis surveillance records were examined in March 2007 in order to identify all locations where *S. japonicum* had been detected after the attainment of transmission control. Reemergence was examined at the smallest community unit, the production group or natural village (referred to here as village), which is typically composed of 100 to 300 residents. Villages were classified as reemerging if schistosomiasis was endemic prior to transmission control and at least one of the following was detected after transmission control: an acute case of human schistosomiasis, *S. japonicum* in a child younger than 12 years or a *S. japonicum*-infected
snail. These reemergence criteria are based on routine post-control surveillance. All acute schistosomiasis cases presenting to health providers are required to be reported to the county schistosomiasis control station. In addition, counties are required to conduct active surveillance for infected snails in a sample of villages twice a year. Human infection surveys are conducted only sporadically after control attainment. County surveillance records were supplemented by provincial surveillance data when available. Twenty-five villages were selected from the surveillance-identified reemerging villages, by county and the method indicating reemergence, for the infection surveys described below.

For comparison, 28 villages were selected from formerly endemic villages with no evidence of reemergence: no infected snails, acute cases of human schistosomiasis or infected children had been detected by county or provincial surveillance since transmission control was attained. As a complete list of all formerly endemic villages in the selected counties does not exist, a convenience sample was drawn.

Census and interviews. In June 2007, surveys were conducted to describe the human and bovine populations in the 53 selected villages. All residents, age six and older, who lived most of the time in the village were invited to complete a brief survey about their age, sex, occupation, highest level of schooling, time spent outside of their village and schistosomiasis treatment history. The head of each household was also asked to complete a longer questionnaire describing household access to water and sanitation, agricultural practices, ownership of domestic animals, socioeconomic indicators and travel. Given the challenges of estimating incomes in agrarian regions [12], household socio-economic status was measured in terms of the number of eight different items owned by members of the household. The head of each household was asked if anyone who lived in the house owned eight different items (a car, tractor, motorcycle, computer, television, washing machine, air conditioner or refrigerator) and a household inventory score was assigned based on the number of items owned. In 2008, attempts were made to interview any participant in the human infection survey missing household or individual interview data from 2007.

All questionnaires were pilot-tested. Interviews were conducted by trained staff at the Institute of Parasitic Diseases (IPD), Sichuan Center for Disease Control and Prevention or the county Anti-schistosomiasis Control stations, fluent in Sichuan dialect which is spoken by the study population. Household and individual interviews were scanned using optical mark recognition software (Remark Office OMR, Gravic Corporation, Malvern, PA). Approximately 10% of scanned questionnaires were checked against paper records to ensure data accuracy.

Infection surveys. Humans, snails and bovines were tested for *S. japonicum* infection in the 53 selected villages in 2007.

In April, all irrigation ditches in each village were surveyed for the intermediate snail host, *Oncomelania hupensis robertsoni*. Teams of trained IPD and County Anti-schistosomiasis Control station staff with extensive experience conducting snail surveys collected samples at ten meter intervals along all irrigation ditches, sampling a total of 15,054 locations. At each location, a square frame (*kuang*) measuring 0.11 m² was placed at the waterline and all *O. hupensis* snails within the frame were collected. In addition, snails were sampled from the base of ten terrace walls per village (or, if fewer than ten terraces were present, all terraces in the village), since the lower portions of terrace walls accumulate moisture and may provide suitable habitat for snails. Using the *kuang* sampling frame, snails were collected from the middle and both ends of the terraces at a total of 2,498 sampling locations. The location of each sampling site was recorded using a handheld GPS unit, allowing for the simultaneous generation of irrigation ditch maps for
each village [13]. Collected snails were deposited in paper envelopes and brought to the laboratory where they were crushed between two glass slides and inspected for cercaria using a dissecting microscope. Of the collected snails, 97.3% were tested for *S. japonicum* infection.

Human infection prevalence and intensity were measured in November and December 2007. All residents age six to 65 were invited to submit three consecutive stool samples which were analyzed using the miracidial hatch test and the Kato-Katz thick smear procedure [14,15]. Samples were collected from villages daily and brought to a central laboratory in each county where they were stored out of direct sunlight until processing. Three stool samples were collected from 2,504 infection survey participants (83%), two samples from 202 people (7%) and one sample from 303 people (10%).

Using the miracidial hatch test, each sample was suspended and, after three and five hours, examined for miracidia. Briefly, approximately 30 grams of stool were suspended in aqueous solution. The sample was first strained with copper mesh to remove large particles, and strained a second time with nylon mesh to concentrate schistosome eggs. This sediment was re-suspended and left in a room with ambient temperatures between 28 and 30 degrees C. Three and five hours later, each sample was examined for the presence of miracidia for at least two minutes each time.

In addition, using the Kato-Katz thick smear procedure, three slides were prepared with 41.5 mg each of homogenized stool from the first sample submitted by each participant. In almost all cases (98.6%), three slides were prepared for each person. Two slides were prepared for 21 people (0.7%), and only one slide was prepared for another 21 people (0.7%). Slides were examined using a dissecting microscope and if any *S. japonicum* eggs were detected, the species and number of eggs was confirmed by a second reader. Infection intensity, in eggs per gram of stool (EPG), was calculated as the total number of *S. japonicum* eggs present on the slides divided by the total sample weight. Stool samples were typically prepared within one day of sample collection: this was true of 90% of hatch tests and 92% of Kato-Katz slides. Slides were stored out of sunlight and read a mean of 3.3 days after preparation (max 23 days). A person was classified as infected if the hatch test was positive or at least one egg was detected using the Kato-Katz technique.

Surveys to assess *S. japonicum* infections in the domestic bovines, water buffalo (*Bubalus bubalis*) and cows (*Bos Taurus*), were conducted at the same time as the human surveys. Everyone who owned bovines in the 53 villages was invited to have his or her animals tested. Stool samples were collected from bovines by keeping the animal in a pen or tied until stool was produced and could be collected. Three samples from three different days were collected from 68% of animals, two from 18% and one from 14%. Each sample was examined using the miracidial hatch test as described above. Due to the rapid hatching of miracidia in the bovine samples and the short survival of the miracidia in the suspension, flasks were examined one, two and four hours after sample preparation. Almost all samples (99%) were processed within one day of collection. Bovines were classified as infected if at least one hatch test was positive for *S. japonicum*. Bovines with at least one positive hatch test were subsequently examined using the Danish Bilharziasis Laboratory (DBL) method in order to estimate infection intensity [16]. Briefly, 5 grams of homogenized stool were washed through a series of three sieves (mesh size: 400 μm, 100 μm, 45 μm). The material in the 45 μm sieve was suspended and left in the dark to sediment. The solution was then centrifuged, the top half of the liquid decanted, and the remaining sediment re-suspended and re-centrifuged in order to obtain 2 mL of solution. A thick smear approach was used to count the eggs on up to 30 slides. Infection
intensity (EPG) was calculated as the total number of eggs divided by the total sample weight: five grams.

**Ethical approval and treatment.** All participants provided written, informed consent before participating in this study. The research protocol was approved by the Sichuan Institutional Review Board, the National Institutes of Health Institutional Review Board and the University of California, Berkeley, Committee for the Protection of Human Subjects. Each person who tested positive for *S. japonicum* was provided treatment with praziquantel by the county Anti-Schistosomiasis Control Station. All bovines testing positive were referred to the county Cattle Department for treatment with praziquantel.

**Statistical analysis.** The distribution of human infections was examined by county, age, sex, occupation, educational attainment, socio-economic status, travel outside of the village and treatment history. Two outcomes were examined: infection status, a binary outcome, and infection intensity, measured as a count of the total number of eggs detected on three Kato-Katz slides. The 42 people with only one or two slides examined were excluded from the infection intensity analysis. We modeled infection intensity as a negative binomial distribution. Because adult and child behaviors as well as immunological responses may vary in ways that affect the distribution of infections in the two populations, children (<18 years) and adults were analyzed separately. As minimal differences in infection patterns were detected, the combined results are presented. Similarly, distribution of bovine infections was examined by county, age, sex and the socio-economic status of the owners. Because cows and water buffalo may differ in terms of exposures and immunology, predictors of infection were examined separately for each bovine type. All infection models accounted for the correlation of infections clustered within villages using generalized estimating equations (GEE) with exchangeable correlation and with inference from robust variance estimates [17,18]. Binary human and bovine infection status were modeled using GEE logistic regression, whereas models of infection intensity were conducted using GEE negative binomial regression.

The performance of schistosomiasis surveillance methods including acute case reporting, as well as surveys for *S. japonicum* infections in snails, bovines and targeted human populations were evaluated in terms of their ability to identify villages where human infections were present. The sensitivity (the proportion of “true positives” that test positive) and specificity (the proportion of “true negatives” that test negative) of each method was calculated using the human infection survey results as the “gold standard.” Villages where at least one person tested positive for *S. japonicum* were classified as true positives, and villages where all human stool samples were negative for *S. japonicum* were classified as true negatives. Estimates of the sensitivity and specificity of provincial and county surveillance records and acute case reporting were weighted to account for the fact that villages were selected based on surveillance record findings. Weights were based on the estimated total number of villages formerly endemic for schistosomiasis in the region (*n*), the number of formerly endemic villages where surveillance records indicated the presence of an acute case of schistosomiasis, a *S. japonicum* infected snail or a *S. japonicum* infected bovine (*np*), and the number of villages where one or more acute schistosomiasis cases were reported (*na*). While *np* and *na* were directly measured, *n* is unknown in the region and was estimated, based on conversations with county schistosomiasis control officers, to be 1,000. The weighting procedure is described in detail in the Appendix. In order to estimate the variability around each point estimate, bootstrapping was used [19]. Villages were randomly sampled with replacement to generate a population with the same number of villages as used to calculate the point estimate. Sensitivity and specificity were estimated using the sampled population. This
Table 1. The indicators of reemergence in 112 villages in three counties where schistosomiasis was detected through county and provincial surveillance after transmission control was attained.

<table>
<thead>
<tr>
<th>Villages where surveillance records indicated…</th>
<th>County 1</th>
<th>County 2</th>
<th>County 3</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute schistosomiasis</td>
<td>16</td>
<td>1</td>
<td>8</td>
<td>25</td>
</tr>
<tr>
<td>Infected snails</td>
<td>47</td>
<td>23</td>
<td>30</td>
<td>100</td>
</tr>
<tr>
<td>Infected children</td>
<td>4</td>
<td>0</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Any of the above</td>
<td>47</td>
<td>24</td>
<td>41</td>
<td>112</td>
</tr>
<tr>
<td>Year attained transmission control</td>
<td>1995</td>
<td>1985</td>
<td>1987</td>
<td></td>
</tr>
<tr>
<td>Year reemergence first detected</td>
<td>2000</td>
<td>2000</td>
<td>1997</td>
<td></td>
</tr>
<tr>
<td>Year reemergence last detected</td>
<td>2006</td>
<td>2005</td>
<td>2004</td>
<td></td>
</tr>
</tbody>
</table>

was repeated 1,000 times and the 2.5th and 97.5th percentile values were used to generate 95% confidence intervals.

The prevalence and intensity of human *S. japonicum* infections at the village-level were compared for each surveillance method. For example, infection prevalence and intensity in villages where acute cases have been reported were compared to villages where they were not reported. As village infection prevalence and intensity were not normally distributed, the non-parametric Mann-Whitney two sample test was used.

Tests of statistical significance were conducted setting $\alpha = 0.05$. Data analysis was conducted using Stata, version 10 (StataCorp, College Station, TX, USA) and R, version 2.9.2 (www.r-project.org).

**Results**

**Surveillance records.** Through the examination of county and provincial surveillance records from three counties, 112 villages were identified where an acute case of human schistosomiasis, a *S. japonicum*-infected snail or a *S. japonicum*-infected child was detected following transmission control. This includes 109 villages identified through county surveillance records and three additional villages, all in County 1, identified through provincial surveillance records. Infected snails were the most frequent indicator of reemergence, detected in 100 villages. Acute cases were detected in 25 villages and infected children in seven villages (Table 1).

Reemergence was detected in County 1 just five years after transmission control criteria were met. Acute cases were most common in County 1, detected in 16 of the 47 villages where reemergence was detected, all within the first four years of reemergence, and infected snails were detected in every village (Figure 1). Schistosomiasis was detected 15 years after transmission control was declared in County 2, through the detection of an infected snail. This occurred the same year that reemergence was detected in County 1, which borders County 2. In County 3, evidence of reemergence was first discovered ten years after transmission control through the detection of infected snails. Subsequently, acute infections were detected in eight of the 41 villages that met reemergence criteria.

**Characteristics of residents in the 53 surveyed villages.** Beginning in June 2007, 4,282 residents, including 1,784 heads of household, were interviewed in 53 villages. In addition, 117 people participated in the infection surveys but not the interviews. The number of
Figure 1. The year reemerging schistosomiasis was first detected in 112 villages where transmission was previously controlled, based on county and provincial surveillance records.

County 1 attained transmission control in 1995, County 2 in 1985 and County 3 in 1987.
participants per village ranged from 30 to 169. As shown in Figure 2, there are few teenagers and young adults in the study population relative to other age groups. Residents reported many people, particularly younger populations, had left the rural areas to find work in cities. There is also a dip in the population corresponding with the birth years 1959 to 1961, the years of the Chinese famine. The famine led to approximately 30 million fewer births than would have been expected in China, in addition to causing approximately 15-30 million premature deaths [20,21].

Rural to urban migration was also common among the individuals that were interviewed: 26.5% of people reported leaving their village for more than one month in the past year (Table 2). This was the case for 55.1% of young adults age 18 to 29, 40.2% of adults at 30 to 39 and 26.3% of adults age 40 to 49, most of who left to work as laborers. Some teenagers, age 12 to 17 years, also reported living outside of their village for more than one month in the past year (20.8%), primarily to attend school.

Farming was the dominant occupation in the study villages: 96.5% of adults (>18 years) reported they worked as farmers. Most families reported year-round farming. Rice and corn were the principal crops during the summer growing season which typically begins in May and ends in September. In the year prior to the survey, 71.3% of the land cultivated in the summer was devoted to rice and 21.4% to corn. Peanuts and vegetables were also planted in small quantities, covering 3.4% and 2.8% of the cultivated land in summer, respectively. A second, winter growing season begins in October and ends in April. Most of the land cultivated in winter was devoted to rapeseed (63.0%) and wheat (34.4%) in the year prior to the survey.

Socio-economic status was modest for many households in the survey. Most adults (64.7%) had no formal schooling beyond elementary school and most individuals (61.3%) lived in a household with two or fewer of the eight items listed in the household inventory. Televisions were the most common item, owned by 94.5% of households, followed by washing machines (55.6%), motorcycles (33.2%) and refrigerators (16.5%). Tractors (2.8%), air conditioners (2.0%), cars (1.7%) and computers (0.5%) were rare.

**Human S. japonicum infection.** In November and December 2007, 3,009 people from 53 villages were tested for S. japonicum infection, including 2,671 people who were interviewed in 2007 and an additional 338 new participants, 221 of whom were subsequently interviewed in 2008. Participation in the infection surveys by those interviewed ranged from 45.2% to 96.6%
Table 2. Characteristics of the study population and participants in the human *S. japonicum* infection surveys.

<table>
<thead>
<tr>
<th></th>
<th>Total population</th>
<th>Tested for <em>S. japonicum</em> infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>County</td>
<td></td>
<td></td>
</tr>
<tr>
<td>County 1</td>
<td>1,798</td>
<td>40.9</td>
</tr>
<tr>
<td>County 2</td>
<td>1,200</td>
<td>27.3</td>
</tr>
<tr>
<td>County 3</td>
<td>1,401</td>
<td>31.8</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>2,158</td>
<td>50.2</td>
</tr>
<tr>
<td>Male</td>
<td>2,138</td>
<td>49.8</td>
</tr>
<tr>
<td>Age</td>
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<td></td>
</tr>
<tr>
<td>&lt;12</td>
<td>332</td>
<td>7.8</td>
</tr>
<tr>
<td>12 - 17</td>
<td>357</td>
<td>8.3</td>
</tr>
<tr>
<td>18 - 29</td>
<td>445</td>
<td>10.4</td>
</tr>
<tr>
<td>30 - 39</td>
<td>899</td>
<td>21.0</td>
</tr>
<tr>
<td>40 - 49</td>
<td>878</td>
<td>20.5</td>
</tr>
<tr>
<td>50+</td>
<td>1,368</td>
<td>32.0</td>
</tr>
<tr>
<td>Occupation*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farmer</td>
<td>3,457</td>
<td>96.5</td>
</tr>
<tr>
<td>Other†</td>
<td>126</td>
<td>3.5</td>
</tr>
<tr>
<td>Educational attainment*</td>
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<td></td>
</tr>
<tr>
<td>Elementary school or less</td>
<td>2,314</td>
<td>64.7</td>
</tr>
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<td>Middle school</td>
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<td>29.7</td>
</tr>
<tr>
<td>High school or more</td>
<td>197</td>
<td>5.5</td>
</tr>
<tr>
<td>Household inventory score</td>
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<td>2 items</td>
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</tr>
<tr>
<td>4+ items</td>
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<td>13.2</td>
</tr>
<tr>
<td>Time out of the village last year</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Did not leave</td>
<td>2,576</td>
<td>65.5</td>
</tr>
<tr>
<td>1 - 30 days</td>
<td>314</td>
<td>8.0</td>
</tr>
<tr>
<td>31+ days</td>
<td>1,040</td>
<td>26.5</td>
</tr>
<tr>
<td>Primary activity out of the village</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Did not leave</td>
<td>2,576</td>
<td>65.3</td>
</tr>
<tr>
<td>Labor</td>
<td>1,003</td>
<td>25.4</td>
</tr>
<tr>
<td>School</td>
<td>253</td>
<td>6.4</td>
</tr>
<tr>
<td>Other‡</td>
<td>114</td>
<td>2.9</td>
</tr>
<tr>
<td>Took praziquantel in the past year</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>2,344</td>
<td>57.7</td>
</tr>
<tr>
<td>Yes</td>
<td>1,717</td>
<td>42.3</td>
</tr>
</tbody>
</table>

*Includes only adults (>18 years).
†Other occupations include laborer (67), student (36), business person (14), government official (6) and fisherman (3).
‡Other activities include agriculture (28) and trade (86).
Figure 3. The distribution of human *S. japonicum* infection prevalence, human infection intensity and bovine infection prevalence in 53 villages.

X No bovines tested, O No bovines present
The one infected snail was detected in village 45
Table 3. Univariate predictors of human *S. japonicum* infection prevalence and intensity.

<table>
<thead>
<tr>
<th></th>
<th><em>S. japonicum</em> infection prevalence</th>
<th></th>
<th><em>S. japonicum</em> infection intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n% inf. OR (95% CI)</td>
<td>mean EPG RR (95% CI)</td>
<td></td>
</tr>
<tr>
<td>County</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>County 1</td>
<td>1,233 8.76 1.00</td>
<td>1,139 2.58 1.00</td>
<td></td>
</tr>
<tr>
<td>County 2</td>
<td>877 7.30 0.78 (0.33 - 1.85)</td>
<td>876 1.34 0.51 (0.16 - 1.68)</td>
<td></td>
</tr>
<tr>
<td>County 3</td>
<td>899 2.56 0.20 (0.08 - 0.53)</td>
<td>897 0.54 0.19 (0.05 - 0.72)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>1,500 6.13 1.00</td>
<td>1,463 1.63 1.00</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1,406 7.04 1.18 (0.91 - 1.53)</td>
<td>1,359 1.59 0.97 (0.40 - 2.38)</td>
<td></td>
</tr>
<tr>
<td>Age*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;12</td>
<td>222 2.70 1.00</td>
<td>217 0.00</td>
<td></td>
</tr>
<tr>
<td>12 - 17</td>
<td>121 4.96 1.86 (0.59 - 5.83)</td>
<td>121 0.66</td>
<td></td>
</tr>
<tr>
<td>18 - 29</td>
<td>181 4.42 1.37 (0.54 - 3.48)</td>
<td>172 0.93</td>
<td></td>
</tr>
<tr>
<td>30 - 39</td>
<td>578 7.09 2.41 (0.98 - 5.94)</td>
<td>559 1.67</td>
<td></td>
</tr>
<tr>
<td>40 - 49</td>
<td>659 6.98 2.39 (0.99 - 5.75)</td>
<td>640 2.84</td>
<td></td>
</tr>
<tr>
<td>50+</td>
<td>1,130 7.43 2.57 (1.10 - 5.99)</td>
<td>1,098 1.42</td>
<td></td>
</tr>
<tr>
<td>test for trend, p-value†</td>
<td>0.009</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Occupation*‡</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farmer</td>
<td>2,502 7.03 1.00</td>
<td>2,425 1.84</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>43 6.98 0.75 (0.32 - 1.77)</td>
<td>41 0.00</td>
<td></td>
</tr>
<tr>
<td>Education‡</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elementary school or less</td>
<td>1,752 7.93 1.00</td>
<td>1,702 2.09 1.00</td>
<td></td>
</tr>
<tr>
<td>Middle school</td>
<td>674 5.19 0.66 (0.47 - 0.93)</td>
<td>651 1.16 0.54 (0.21 - 1.35)</td>
<td></td>
</tr>
<tr>
<td>High school or more</td>
<td>110 3.64 0.38 (0.15 - 0.94)</td>
<td>104 0.77 0.33 (0.12 - 0.90)</td>
<td></td>
</tr>
<tr>
<td>test for trend, p-value†</td>
<td>0.011</td>
<td>0.048</td>
<td></td>
</tr>
<tr>
<td>Household inventory score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 - 1 items</td>
<td>869 8.17 1.00</td>
<td>852 1.48 1.00</td>
<td></td>
</tr>
<tr>
<td>2 items</td>
<td>904 7.08 1.02 (0.64 - 1.64)</td>
<td>876 2.27 1.59 (0.57 - 4.47)</td>
<td></td>
</tr>
<tr>
<td>3 items</td>
<td>697 5.16 0.79 (0.46 - 1.36)</td>
<td>673 1.15 0.75 (0.27 - 2.07)</td>
<td></td>
</tr>
<tr>
<td>4+ items</td>
<td>369 4.88 0.87 (0.51 - 1.47)</td>
<td>354 0.86 0.73 (0.18 - 2.87)</td>
<td></td>
</tr>
<tr>
<td>test for trend, p-value†</td>
<td>0.379</td>
<td>0.402</td>
<td></td>
</tr>
<tr>
<td>Time out of the village last year</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Did not leave</td>
<td>1,869 6.37 1.00</td>
<td>1,834 1.11 1.00</td>
<td></td>
</tr>
<tr>
<td>1 - 30 days</td>
<td>144 6.94 1.34 (0.81 - 2.24)</td>
<td>140 1.03 0.79 (0.23 - 2.67)</td>
<td></td>
</tr>
<tr>
<td>31+ days</td>
<td>577 6.07 0.87 (0.63 - 1.20)</td>
<td>539 2.27 1.97 (0.47 - 8.34)</td>
<td></td>
</tr>
<tr>
<td>Primary activity outside village*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Don't go out</td>
<td>1,869 6.37 1.00</td>
<td>1,834 1.11</td>
<td></td>
</tr>
<tr>
<td>Labor</td>
<td>565 6.37 0.96 (0.69 - 1.32)</td>
<td>530 2.47</td>
<td></td>
</tr>
<tr>
<td>School</td>
<td>88 5.68 1.03 (0.46 - 2.31)</td>
<td>85 0.00</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>73 6.85 1.13 (0.56 - 2.30)</td>
<td>70 0.80</td>
<td></td>
</tr>
<tr>
<td>Took praziquantel in the past year</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1,476 4.54 1.00</td>
<td>1,461 0.46 1.00</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1,195 8.70 1.32 (0.96 - 1.80)</td>
<td>1,133 2.62 5.44 (1.88 - 15.72)</td>
<td></td>
</tr>
</tbody>
</table>

*Models of *S. japonicum* infection intensity could not be fit because in some groups no eggs were detected.
†Test for trend was performed treating categories as ordinal.
‡Includes only adults (>18 years).

EPG = Eggs per gram of stool
by village and varied by county, age, occupation and travel out of the village (Table 2). Participation was lowest for teenagers and students and was also low for young adults and laborers, populations which overlap considerably.

Human infections were detected in 35 villages. Mean human infection prevalence was 6.5% and ranged from 0 to 42.9% by village (Figure 3). Mean infection intensity was 1.6 EPG, with a maximum village infection intensity of 10.6 EPG. Most *S. japonicum* eggs were clustered in a few individuals. Of the 195 individuals who tested positive for *S. japonicum*, only 88 had any detectable eggs, and 24% of the total eggs detected were excreted by two individuals.

The prevalence and intensity of infections varied by county of residence, age and recent praziquantel treatment (Table 3). The prevalence and intensity of infections in Counties 1 and 2 were similar, but County 3 had lower levels of infection. Infection prevalence and intensity increased with age (Figure 4). Children were least likely to be infected and no children younger than 12 years had eggs in their stool – the six infected children tested positive for *S. japonicum* by the miracidial hatch test only. Infection prevalence appeared to plateau at ages 30 to 39, whereas infection intensity was highest among those 40 to 49 and decreased slightly in the oldest age groups. While infection prevalence did not vary by sex among adults, boys (<18 years) were significantly more likely to be infected than girls (infection prevalence 5.0% vs. 1.9%, OR 2.89, 95% CI: 1.13 – 7.36). Adults with more formal schooling were less likely to be infected and had lower infection intensities than adults with no more than an elementary school education. Infection was not associated with household socio-economic status, time spent out of the village or activities conducted outside of the village. Almost half of participants (42.3%) reported taking the anti-helminthic drug, praziquantel in the past year and those who did had infection intensities five times higher than those who had not been treated recently.

**Bovine *S. japonicum* infection.** In November 2007, research teams identified 821 bovines in 50 villages and 537 (65.4%) of these bovines from 44 villages were tested for *S. japonicum* infection. The six villages where bovines were present but infection surveys were not conducted all had fewer than ten bovines (mean 6.0), whereas villages where infection surveys were conducted had a mean of 16.9 bovines. The number and type of bovines varied by county.
Table 4. Characteristics of the 821 bovines identified in 50 villages and those that participated in *S. japonicum* infection surveys.

<table>
<thead>
<tr>
<th>County</th>
<th>No.</th>
<th>%</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>County 1</td>
<td>360</td>
<td>43.8</td>
<td>213</td>
<td>59.2</td>
</tr>
<tr>
<td>County 2</td>
<td>359</td>
<td>43.7</td>
<td>290</td>
<td>80.8</td>
</tr>
<tr>
<td>County 3</td>
<td>102</td>
<td>12.4</td>
<td>34</td>
<td>33.3</td>
</tr>
</tbody>
</table>

Type

<table>
<thead>
<tr>
<th>Type</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cow</td>
<td>560</td>
<td>69.7</td>
</tr>
<tr>
<td>Water buffalo</td>
<td>243</td>
<td>30.3</td>
</tr>
</tbody>
</table>

Sex*

<table>
<thead>
<tr>
<th>Sex*</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>411</td>
<td>88.0</td>
</tr>
<tr>
<td>Male</td>
<td>56</td>
<td>12.0</td>
</tr>
</tbody>
</table>

Age*

<table>
<thead>
<tr>
<th>Age*</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 2 years</td>
<td>129</td>
<td>28.0</td>
</tr>
<tr>
<td>2.1 - 4 years</td>
<td>178</td>
<td>38.7</td>
</tr>
<tr>
<td>&gt; 4 years</td>
<td>153</td>
<td>33.3</td>
</tr>
</tbody>
</table>

Household inventory score

<table>
<thead>
<tr>
<th>Household inventory score</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 1 items</td>
<td>381</td>
<td>48.2</td>
</tr>
<tr>
<td>2 items</td>
<td>226</td>
<td>28.6</td>
</tr>
<tr>
<td>3 items</td>
<td>131</td>
<td>16.6</td>
</tr>
<tr>
<td>4+ items</td>
<td>52</td>
<td>6.6</td>
</tr>
</tbody>
</table>

*Age and sex were not collected for 361 and 354 bovines, respectively. 91 (25.2%) bovines without age data and 123 (34.7%) bovines without sex data were tested for *S. japonicum* infection.

Nearly all bovines in County 2 were cows (95.5%). In County 1, which had almost the same number of bovines as County 1, 56.6% were cows. There were fewer bovines in County 3 – all three of the villages without any bovines were located in this county – and only 25.5% of these bovines were cows. Participation in the bovine infection surveys varied by county and bovine type.

Bovine infections were detected in 23 villages. Mean bovine infection prevalence was 13.4%, ranging from 0 to 65.4% by village (Figure 3). Of the 72 bovines that tested hatch positive, 67 were examined to assess infection intensity and 11 had any detectable eggs. While bovine infection prevalence was higher than human infection prevalence, bovine infection intensity was lower, averaging 0.015 EPG. The highest mean village infection intensity in bovines was 0.11 EPG.

There were few strong determinants of bovine infection prevalence (Table 5). Infection prevalence was modestly but not significantly higher in cows compared to water buffalo (14.25% vs. 10.26%, p=0.376). Infection prevalence declined sharply with age for water buffalo: each year of age was associated with an approximate halving of infection probability (OR 0.57, 95% CI 0.32 – 0.99) (Figure 5). In contrast, cow infection prevalence increased slightly with each year of age (OR 1.06, 95% CI 0.99 – 1.13). Bovine infection prevalence was lowest in County 1,
Table 5. Univariate predictors of *S. japonicum* infection in 407 cows and 117 water buffalo.

<table>
<thead>
<tr>
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<th>Cows</th>
<th>Water buffalo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>% inf.</td>
</tr>
<tr>
<td>County</td>
<td></td>
<td></td>
</tr>
<tr>
<td>County 1</td>
<td>121</td>
<td>8.26</td>
</tr>
<tr>
<td>County 2</td>
<td>279</td>
<td>16.49</td>
</tr>
<tr>
<td>County 3</td>
<td>7</td>
<td>28.57</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>304</td>
<td>15.79</td>
</tr>
<tr>
<td>Male</td>
<td>41</td>
<td>19.51</td>
</tr>
<tr>
<td>Age*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 2 years</td>
<td>87</td>
<td>11.49</td>
</tr>
<tr>
<td>2.1 - 4 years</td>
<td>134</td>
<td>11.19</td>
</tr>
<tr>
<td>&gt; 4 years</td>
<td>130</td>
<td>22.31</td>
</tr>
<tr>
<td></td>
<td>test for trend, p-value†</td>
<td>0.048</td>
</tr>
<tr>
<td>Household inventory score*</td>
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<td></td>
</tr>
<tr>
<td>0 - 1 items</td>
<td>228</td>
<td>11.84</td>
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<tr>
<td>2 items</td>
<td>111</td>
<td>18.92</td>
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<td>19.44</td>
</tr>
<tr>
<td>4+ items</td>
<td>12</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>test for trend, p-value†</td>
<td>0.032</td>
</tr>
</tbody>
</table>

*Some models could not be fit because no infections or no eggs were detected on one or more groups.
†Test for trend was performed treating categories as ordinal.

where human infection prevalence was greatest. Water buffalo infection prevalence was highest in households with the highest household inventory scores, a pattern not observed for cows. Bovine infections did not vary by the sex of the animal.

**Snail *S. japonicum* infection.** *O. hupensis* were present in 38 of the 53 villages surveyed. Snails were present in irrigation ditches in 34 villages and along terrace walls in 17 villages. Villages averaged 2.3 snails/m² in irrigation ditches (range: 0 to 24.8 snails/m²). Out of 7,325 snails collected from irrigation ditches, one infected snail was found. Mean snail density along terraces was 0.6 snails/m² (range: 0 – 8.5 snails/m²). None of the 190 snails collected from terrace walls were infected. Snail densities in terraces were not strongly correlated with snail densities in irrigation ditches (Spearman’s rho 0.302).

**Evaluation of surveillance strategies.** County and provincial surveillance records were a poor indicator of the presence of human infection in a village. Human infections were detected in 18 of the 25 villages where county and provincial surveillance records had indicated reemergence, and in 17 of the 28 villages where reemergence had not previously been detected. Assuming there were 1,000 formerly endemic villages in the three counties, the 112 villages identified through surveillance records represent 11.2% of all formerly endemic villages. Assigning a weight of 11.2% to the surveillance positive villages and 88.8% to the surveillance negative villages, the estimated sensitivity of county and provincial surveillance records for detecting the presence of human *S. japonicum* infections in a village was 13% and specificity was 92% (Table 6).
Figure 5. The prevalence of *S. japonicum* infections in water buffalo and cows by age. Note that no water buffalo over four years old were infected.

Acute case reporting, one component of the county and surveillance records, also yielded low sensitivity. Human infections were detected in nine of the 11 villages where acute schistosomiasis had been reported and 26 of the 42 villages where acute schistosomiasis had not been reported. Acute schistosomiasis was reported in 25 of the 112 surveillance positive villages identified in the three counties. Using this information to reweight the distribution of villages (described in detail in the Appendix), the estimated sensitivity of acute case reporting was 3% and the specificity was 99%. Despite their poor performance in identifying villages where human *S. japonicum* infections were present, infection prevalence and intensity were higher in villages where acute cases had been detected (prevalence 13.7% vs. 5.1%, p=0.011; intensity 4.1 EPG vs. 0.8 EPG, p=0.005), and in villages where there was any indication of reemergence (prevalence 10.3% vs. 3.8%, p=0.030; intensity 2.2 EPG vs. 0.9 EPG, p=0.042)

The presence of *S. japonicum*-infected snails was a poor indicator of the presence of human schistosomiasis since only one infected snail was detected in the 53 villages. The sensitivity of this method was 3% (95% CI 0 – 9%). Surveys that simply looked for the presence of *O. hupensis*, regardless of infection status, had better sensitivity: 69% when irrigation ditches were surveyed and 74% when irrigation ditches and terraces were surveyed. However the specificity of snail surveys in irrigation ditches or irrigation ditches and terraces was low: 44% and 33%, respectively.

Bovines were present in 34 of the 35 villages with human schistosomiasis, thus the presence of bovines has high sensitivity (97%). But specificity was low (11%) as bovines were also present in 16 of the 18 villages without human infections. Testing bovines for *S. japonicum* infections had sensitivity of 59%, slightly lower than the sensitivity of infected snail surveys conducted in irrigation ditches. The specificity was slightly higher (67%) than that of infected snail surveys. Human infection prevalence was higher in villages with infected bovines (10.7% vs. 5.0%, p=0.022) but infection intensity did not vary significantly between the two groups (2.5 EPG vs. 1.0 EPG, p=0.064). Infection prevalence and intensity were not significantly higher in villages where bovines were present.
Table 6. The sensitivity and specificity of surveillance methods to detect the presence of human *S. japonicum* infections in a village.

<table>
<thead>
<tr>
<th>Human infections*</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Surveillance records indicate reemergence†‡</td>
<td>18</td>
<td>7</td>
</tr>
<tr>
<td>No</td>
<td>17</td>
<td>11</td>
</tr>
<tr>
<td>Acute schistosomiasis reported†</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>No</td>
<td>26</td>
<td>16</td>
</tr>
<tr>
<td><em>S. japonicum</em> infections in <em>O. hupensis</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>No</td>
<td>34</td>
<td>18</td>
</tr>
<tr>
<td><em>O. hupensis</em> in irrigation ditches</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>24</td>
<td>10</td>
</tr>
<tr>
<td>No</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td><em>O. hupensis</em> in terraces</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>No</td>
<td>25</td>
<td>11</td>
</tr>
<tr>
<td><em>O. hupensis</em> in irrigation ditches or terraces</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>26</td>
<td>12</td>
</tr>
<tr>
<td>No</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>Bovines present in village</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>34</td>
<td>16</td>
</tr>
<tr>
<td>No</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td><em>S. japonicum</em> infections in bovines</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>19</td>
<td>4</td>
</tr>
<tr>
<td>No</td>
<td>13</td>
<td>8</td>
</tr>
<tr>
<td>Infections in children &lt;18 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>No</td>
<td>26</td>
<td>17</td>
</tr>
<tr>
<td>Infections in people age 30 - 49 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>28</td>
<td>0</td>
</tr>
<tr>
<td>No</td>
<td>7</td>
<td>18</td>
</tr>
</tbody>
</table>

*Village-wide infection surveys were used as the gold standard. Every resident was asked to provide three stool samples which were examined using the miracidial hatch test and Kato-Katz thick smear procedure. Villages were classified as positive for human infections if at least one person tested positive for *S. japonicum* infection.

†Estimates of sensitivity and specificity were weighted to account for oversampling of villages where surveillance records indicated reemergence.

‡Reemergence was indicated by the reporting of an acute case of human schistosomiasis, or the detection of *S. japonicum*-infected snails or *S. japonicum*-infected children by county or provincial disease control teams after the attainment of transmission control.
Targeted surveys of humans age 30 to 49 yielded high sensitivity (80%), and the method was superior to targeted sampling of children (sensitivity 26%). The specificities of targeted human surveillance methods are, by definition, 100%.

Discussion

Human *S. japonicum* infection prevalence was 6.5% and bovine infection prevalence was 13.4% in 53 villages where the transmission of schistosomiasis had previously been controlled, indicating widespread reemergence of schistosomiasis in the region. Surveillance strategies that relied on acute schistosomiasis case reporting alone or in combination with county and provincial surveys for infected snails and children grossly underestimated the number of villages with human schistosomiasis. Human infections were detected in 17 of 28 villages where county and provincial surveillance records provided no prior indication of reemergence. Similarly, surveys for *S. japonicum*-infected snails carried out by this research team identified only one infected snail out of over 7,000 snails sampled, indicating surveys for infected snails are not a reliable surveillance method for monitoring the reemergence of human schistosomiasis in this region. Targeted testing of humans, particularly adults age 30 to 49, yielded high sensitivity. While labor intensive, human infection surveys appear to be the most reliable indicator of the return of human infections following control.

There is a need for accurate, cost-effective post-schistosomiasis control surveillance strategies that can promptly identify the return of human infections. The fact that human infection prevalence exceeded 20% in six villages suggests transmission of human infections had been ongoing for an extended period of time. Further, Counties 1 and 2, which share a border, reemerged in the same year, and several villages with high human infection prevalence were located near each other on either side of the county line, suggesting inter-village connections may amplify the area of reemergence. Timely detection of infections may allow for the containment of transmission. Schistosomiasis, like many infectious diseases, does not conform to administrative boundaries and a surveillance system that promotes regional collaboration is needed.

Historically, the detection of acute schistosomiasis cases have served as sentinel events indicating the emergence of schistosomiasis in new areas and reemergence in previously endemic areas [6,7]. Due to the severity of symptoms that arise shortly after infection, acute schistosomiasis, reporting provides an inexpensive, timely method of tracking human schistosomiasis. But acute schistosomiasis is rare, comprising less than 1% of all schistosomiasis cases in China [22] and this study estimated the sensitivity of this method to be only 3%, suggesting the number of villages where schistosomiasis has reemerged may be underestimated by a factor of 30 when acute case surveillance is the sole method used to monitor reemergence. It is important to note that the estimated sensitivity and sensitivity of this method are based on an approximation of the number of endemic villages in the region (1,000), as the precise number is unknown. This approximation is likely an underestimate, indicating that the true sensitivity may be even lower. Most cases of acute schistosomiasis are observed in individuals less than 20 [7,22], thus acute schistosomiasis case reporting relies primarily on children and teenagers to present with this severe, sentinel disease.

Surveillance methods that can identify *S. japonicum* in environmental media or non-human hosts have the potential to identify reemergence before people become infected. But none of the non-human monitoring strategies evaluated here yielded adequate sensitivity and
specificity. Surveys for *S. japonicum* infections in the snail host, *O. hupensis*, have been a central post-control surveillance strategy in the five provinces where schistosomiasis transmission has been interrupted in China and have been recommended in other regions nearing the elimination of transmission [5,23]. However, this research team found only one infected snail out of over 7,000 examined, yielding a sensitivity of only 3%. Because only one infected snail was detected, the snail survey was repeated in half of the study villages in September 2007. Again, only one infected snail was detected. The study region has been subject to intense snail control efforts – in the year before the survey, molluscicide was applied to snail habitats, particularly irrigation ditches, from one to three times – and, likely as a result, snail densities are lower than have been observed elsewhere in Sichuan [24]. In addition, human and bovine infection intensities were also low, suggesting few schistosome eggs are being released into the environment, and therefore snail infections should be limited.

While surveys for infected snails are not an effective surveillance strategy in this region, surveys for the presence of the snail host yielded a more satisfactory sensitivity: 69%. As the specificity of this method was low (44%), it would need to be supplemented with a secondary surveillance method. Surveys for the presence of snails could be used, for example, to identify areas for direct testing of human populations.

Bovine infection surveys had lower sensitivity but higher specificity than surveys for the presence of *O. hupensis*. Collecting three stool samples from every bovine in a village, as well as processing the samples are more costly and labor intensive than snail surveys. Bovine infection surveys could be more desirable if a high-risk population were identified, limiting the target population. I found older water buffalo had minimal infection risk, a phenomenon which has been observed elsewhere [25-27]. Water buffalo appear to have greater resistance to infection than cows – they have lower rates of worm establishment, longer pre-patent periods (the time between infection and the initiation of egg laying), and excrete eggs for shorter periods than cows following infection [28]. The age-related infection patterns observed here may be the result of age or exposure-acquired immunity. However, because cows greatly outnumbered water buffalo in the study region, removing older water buffalo from the target population yielded only minimal reductions in the number of animals targeted for testing. In areas where water buffalo are more numerous, infection surveys in young water buffalo and cows may be desirable.

Human infection surveys are the most precise surveillance strategy as they directly measure the distribution of human schistosomiasis. But, like bovine infection surveys, they are costly and labor intensive. The identification of a high-risk human population for targeted infection surveys based on individual demographics or geographical characteristics could focus human surveillance and make the method more efficient and feasible. Human infection risk increased with age, a phenomenon that has been observed previously in China [29,30]. Children, due to their low infection prevalence, were poor surveillance targets. Infection surveys targeting adults age 30 to 49 yielded high sensitivity (80%) and, as it included 43% of the total population, this method reduced the number of infection tests needed by over half compared to population-wide sampling. Infection surveys targeting all adults age 30 and over yielded sensitivity of 97%, but this included 82% of the total population, thus the efficiency gains are minimal. Work to further refine the definition of high-risk populations for infection based on water contact and travel patterns is currently underway.

While age was a significant predictor of infection status, few other demographic characteristics were associated with infection. In contrast, human and bovine infections varied
markedly by village and county. County-level administration of schistosomiasis control activities may explain some of the geographic variability observed in this study. But the large village-level variation in the distribution of both human and bovine infections, even within counties, suggests the importance of village-level environmental factors in promoting schistosomiasis infection. Liang et al. define the suitability of local environments for schistosomiasis transmission as *internal potential* and have demonstrated changes to village-level conditions, such as sanitation, can yield reductions in human infections [31]. The factors that define internal potential could also be used to identify high-risk areas for infection and focus surveillance. The role of specific local environments in promoting human and bovine infections in the reemerging context is examined in detail in Chapter 4.

Further motivation to identify high-risk environments for schistosomiasis reemergence is provided by the surprising observed relationship between praziquantel and human infection. Approximately half of participants reported taking praziquantel in the past year. Infection prevalence and intensity were higher in populations who reported recent treatment. This finding may indicate chemotherapy is being targeted at individuals who are at high risk of infection. But it may also indicate non-compliance with treatment, perhaps by not taking all of the treatment provided, treatment failure or high levels of reinfection. This troubling finding suggests the effectiveness of chemotherapy-based control efforts may be compromised and supplemental intervention strategies may be needed to reduce infections.

While bovines do not appear to be an ideal surveillance target in the study region, they may play an important role in the reemergence of human schistosomiasis. Bovines are an important driver of endemic schistosomiasis in the lakes and marshland regions of China [25,32], and recent attention has focused on their removal as a disease-control measure [33]. In the reemerging context, bovines may enhance a village’s internal potential due to their potentially large output of schistosome eggs, or may move parasites into previously controlled areas. Water buffalo excrete, on average, 100 times more stool per day than humans and cows produce approximately 40 times more stool than humans [34]. In the study villages, bovine infection intensity was approximately ten times lower than human infection intensity, indicating that daily egg output by humans and bovines may be similar in this region. As bovines are often traded between villages (47% of households reported buying their bovines from another village), they may move parasites between endemic and controlled areas, initiating reemergence. Infected bovines were present in 19 of the 35 villages where human infections were detected and human infection prevalence was higher in villages with infected bovines. While these results suggest bovine and human schistosomiasis may be linked, it is difficult to estimate the contribution of bovines to human schistosomiasis in the reemerging context, a topic that is explored further in Chapter 4.

The snail surveys demonstrated that agricultural terraces can provide suitable habitat for snails. No infected snails were found in terraces, but given the scarcity of infected snails, it is unclear if this is because snail infections are unlikely in the terrace environment or simply due to the low prevalence of snail *S. japonicum* infections in this population. Whether snails actually become infected in the terrace environment and release cercaria into tiny streams or puddles, leading to mammalian infections, or provide a reservoir of snails that populate nearby irrigation ditches and ponds, areas more typically associated with snail to mammal schistosomiasis transmission, remains unclear and warrants further investigation.

Many people reported leaving their villages for extended periods of time to find work or attend school, but infection prevalence did not vary based on the amount of time spent outside of
the village. It is possible that even those who spent months outside of the village may return for key agricultural events such as planting and harvest, activities that may involve cercaria exposure. It is possible that such populations may miss schistosomiasis treatment campaigns, and therefore may serve as a reservoir of infections for their village. Although infection prevalence and intensity among children were low, boys were significantly more likely to be infected than girls, possibly reflecting differences in water contact patterns – boys may have more agricultural duties than girls or be more likely to swim or splash in irrigation channels or ponds.

In interpreting the findings presented here, it is important to consider several potential limitations. The gold standard against which surveillance techniques were assessed is imperfect, as is often the case. While efforts were made to test everyone in the 53 selected villages for infection, approximately 30% of the population did not participate in the infection surveys and participation is likely underestimated, as some individuals and households were missed by the census conducted in the summer of 2007. Estimating the true population of each of the study villages, and therefore the true infection survey participation percentage, is difficult. Due to residency requirements, government population registers in rural areas often include families that have moved to urban areas without registering such moves. Conversations with village leaders suggest almost all residents who spent most of their time in the village were captured by the demographic and household surveys. Participation in the infection surveys was lowest among people who spent time out of the village in the past year and young adults. While, neither of these populations appeared to be at high risk of schistosomiasis, the possibility that some villages had infections that were not detected cannot be ruled out. In addition, while Kato-Katz and the miracidial hatch test are widely recommended methods for the diagnosis of schistosomiasis and the methods are highly specific, they do not detect all infections. The use of multiple stool samples, the preparation of multiple Kato-Katz slides and the combination of two diagnostic tests has been shown to improve sensitivity of these methods. However, at low infection intensities the sensitivity of the hatch test is reduced [35].

The sensitivities and specificities calculated here are for the methods as described in the methods section and adaptations of these methods may result in altered performance. For example, surveys for the presence of *O. hupensis* in irrigation ditches were conducted by sampling all irrigation ditches in a village at ten meter intervals. If only some irrigation ditches are sampled, the probability of finding the snail host, and therefore the sensitivity of the test may be diminished. Similarly, immuno-assays are often used to screen individuals and individuals with a positive immuno-assay result are then asked to provide a stool sample for examination using the hatch or Kato-Katz test, as was done in the 2004 national schistosomiasis survey [1]. Targeted sampling of high risk populations, such as adults age 30 to 49, using an immuno-assay based method will yield different sensitivities than those calculated here. While the sensitivity of some immuno-assay methods has been estimated to be 83 to 96% for the diagnosis of individual infection [36], this research team has had trouble attaining similar sensitivities in field-based infection surveys. The performance of these surveillance methods may vary regionally and may be different in endemic regions that have not yet met transmission control criteria. Identification of human target groups, in particular, may be site specific as, in some regions, infection is closely tied to occupational exposures through fishing and in this study region, few individuals reported fishing as their occupation [37]. More research is needed to evaluate the performance of the surveillance strategies presented here in different *S. japonicum* endemic regions and to examine how they may be applied to post-control surveillance for *S. mansoni* and *S. haematobium*. 
The reduction of human schistosomiasis in China has been remarkable, but also introduces a new set of post-control challenges. Human and bovine infection prevalence in the previously controlled study area were substantial despite recent snail control efforts and treatment of almost half of participants with praziquantel in the past year. Ideally, an infectious disease that is the target of eradication lacks non-human reservoirs, interventions are available to interrupt transmission and the infection can be diagnosed with high sensitivity and specificity [38]. The numerous non-human reservoirs for *S. japonicum* and the fact that, while praziquantel cures over 90% of infections at low cost and with minimal side effects, reinfection following treatment is common, make the elimination of human schistosomiasis in China particularly challenging. Delays in the global eradication of poliomyelitis, a vaccine preventable infection without non-human reservoirs, demonstrates the difficulty of controlling even diseases that are more ideal eradication candidates [39]. Sustaining schistosomiasis control achievements requires a post-control surveillance system that is able to identify areas where schistosomiasis has reemerged in order to enable rapid response to such setbacks and prevent further lapses in control. Acute case surveillance and surveys for *S. japonicum*-infected snails, two commonly used surveillance methods, had very low sensitivities, greatly underestimating the extent of reemergence and appear to be ineffective surveillance tools in post-control areas. While human infection surveys are resource intensive, they may present the best means of monitoring reemergence in post-control environments. Similar strategies are being used to monitor lymphatic filariasis following control [40]. The identification of high risk individuals for infection as presented here, and high risk environments for reemergence, a topic explored in the next chapter, can aid in focusing human surveillance efforts, enabling the refinement of an efficient and effective method for identifying areas where schistosomiasis has returned, without which disease control achievements are endangered.

References


Appendix to Chapter 3

The estimation of the sensitivity and specificity of county and provincial surveillance records and of acute case reporting must account for the method used to select study villages. Villages were selected based on surveillance records, selecting 25 villages formerly endemic for schistosomiasis where acute schistosomiasis, *S. japonicum*-infected snails or *S. japonicum*-infected children had been detected by county or provincial surveillance systems following the attainment of transmission control (surveillance positive). In addition, 28 formerly endemic villages were selected where no such evidence of reemergence had been found (surveillance negative). This sampling strategy leads to an oversampling of surveillance positive villages, biasing estimates of the sensitivity and specificity of this surveillance method.

The sample can be reweighted to account for the actual distribution of surveillance positive and negative villages in the region if the total number of formerly endemic villages (*n*) and the total number of surveillance positive villages (*n_p*) are known.

**Table 7.** Observed distribution of villages, using surveillance-based selection of villages.

<table>
<thead>
<tr>
<th>Surveillance records indicate reemergence</th>
<th>Human infections</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>No</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Village selection</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>a+b</td>
<td>a</td>
</tr>
<tr>
<td>Negative</td>
<td>c+d</td>
<td>c</td>
</tr>
</tbody>
</table>

Using the notation shown in Table 7, the observed sample can be reweighted to account for the oversampling of surveillance positive villages, as shown in Table 8.

**Table 8.** Reweighted distribution of surveillance positive and negative villages, used to calculate sensitivity and specificity.

<table>
<thead>
<tr>
<th>Surveillance records indicate reemergence</th>
<th>No. villages</th>
<th>Human infections</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>n_p</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>n - n_p</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>d</td>
<td></td>
</tr>
</tbody>
</table>

These reweighted values can be used to calculate unbiased estimates of sensitivity and specificity of county and provincial surveillance records.

Similarly, reweighting can be used to estimate the sensitivity and specificity of acute case reporting. Because acute cases were reported in a fraction of all surveillance positive villages, a second weight is required to rebalance the population. The total number of villages where acute cases were reported (*n_a*) can be used to estimate the percent of surveillance positive villages where acute cases were detected (*n_a/n_p*). Villages where no acute cases were detected are divided into two groups to distinguish surveillance positive and negative villages (Table 9).
Table 9. Observed distribution of villages where acute cases were reported, based on surveillance-based selection of villages.

<table>
<thead>
<tr>
<th>Acute schistosomiasis reported</th>
<th>No. villages</th>
<th>Human infections</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>a + b</td>
<td>a</td>
</tr>
<tr>
<td>No, surveillance positive</td>
<td>c + d</td>
<td>c</td>
</tr>
<tr>
<td>No, surveillance negative</td>
<td>e + f</td>
<td>e</td>
</tr>
</tbody>
</table>

This sample can be reweighted to account for the oversampling of surveillance positive villages (Table 10).

Table 10. Reweighted distribution of villages where acute cases were reported.

<table>
<thead>
<tr>
<th>Acute schistosomiasis reported</th>
<th>No. villages</th>
<th>Human infections</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>n_a</td>
<td>n_a (a + b)</td>
</tr>
<tr>
<td>No, surveillance positive</td>
<td>n_p - n_a</td>
<td>(n_p - n_a) c</td>
</tr>
<tr>
<td>No, surveillance negative</td>
<td>n - n_p</td>
<td>(n - n_p) e</td>
</tr>
</tbody>
</table>

Sensitivity can be calculated combining the false negatives:

\[
\frac{n_a \frac{a}{(a + b)}}{n_a \frac{a}{(a + b)} + (n_p - n_a) \frac{c}{(c + d)} + (n - n_p) \frac{e}{(e + f)}}.
\]

Similarly, specificity can be calculated combining the true negatives:

\[
\frac{(n-n_p) \frac{f}{(e+f)}}{n_a \frac{b}{(a + b)} + (n_p - n_a) \frac{d}{(c + d)} + (n - n_p) \frac{f}{(e + f)}}.
\]

When bootstrapping was used to estimate confidence intervals around each sensitivity and specificity estimate, n, n_p and n_a were treated as fixed values.
CHAPTER 4

Defining high-risk environments for human and bovine Schistosoma Japonicum infection in areas where schistosomiasis has reemerged

Introduction

Large declines in human schistosomiasis have been attained by national and international schistosomiasis control programs, primarily through the distribution of the antihelminthic drug, praziquantel [1-3], but an increase in human infections following cessation of such control programs has been observed worldwide [1,4-6]. The prevalence and intensity of schistosomiasis often varies greatly even within relatively small areas, as observed in Chapter 2 and elsewhere, suggesting local environments may modulate endemic schistosomiasis transmission [7,8]. These same local conditions, factors that facilitate the distribution of schistosome eggs, define snail habitats and promote human water contact, may also favor the reemergence of schistosomiasis, defined here as the return of human infections after they have been reduced below detectable levels. Mathematical models have demonstrated sustained reductions in human schistosomiasis when praziquantel treatment is coupled with interventions that alter local environmental conditions, specifically, interventions that reduce the distribution of schistosome eggs through improvements in sanitation or the elimination of bovine infections [9,10]. However, epidemiological evidence from areas where schistosomiasis has reemerged to support these findings is lacking. The long-term success of schistosomiasis control programs may hinge on the ability to identify and alter high-risk environments for reemergence. Here, the impact of conditions that promote the distribution of schistosome eggs from mammalian hosts on the reemergence of schistosomiasis is considered.

The dispersal of schistosome eggs from human hosts can theoretically be controlled through improvements in sanitation, but to date, observational and experimental evidence is limited. Two controlled studies have shown a decrease in intestinal schistosomiasis in villages where latrines have been constructed, relative to control villages, but in both cases, latrine construction was one part of a multi-faceted intervention and thus, the direct impact of sanitation improvements cannot be estimated [11,12]. Observational studies of the effect of sanitation on schistosomiasis transmission have yielded conflicting results and failed to control for potential confounders, such as socio-economic status (SES) [13,14]. In China, the potential protective effect of sanitation is complicated by the use of human waste as an agricultural fertilizer, which is typically extracted from latrines. The impact of fertilization with human waste, or night soil, on schistosomiasis transmission has received little attention.

S. japonicum is a zoonosis with at least 40 competent mammalian hosts in China [15]. The presence of non-human reservoirs may also facilitate the distribution of S. japonicum eggs.
Both cows and water buffalo produce a large volume of stool and have the potential to contribute numerous schistosome eggs relative to other wild and domestic hosts [16]. Bovines have been shown to play a key role in human *S. japonicum* infections in the lakes and marshland regions of the Yangtze River Valley where schistosomiasis is endemic. Experimental studies have shown regular treatment of bovine schistosomiasis, when accompanied by human chemotherapy, yields greater declines in human infections than treatment of humans alone [17-19]. The role of bovines in the transmission of schistosomiasis in the mountainous regions of Sichuan Province remains unknown, as does the contribution of bovines to the reemergence of schistosomiasis.

In this chapter, I examine three conditions that may impact the dispersal of schistosome eggs on mammalian schistosomiasis infection in a region where the disease has reemerged: access to sanitation, the use of human waste as an agricultural fertilizer and the presence of bovines. A cross-sectional study conducted in 53 villages is used to test the hypothesis that village-level night soil use, sanitation coverage and bovine density predict human *S. japonicum* infection. In addition, I examine night soil use, sanitation and bovine density as predictors of bovine infection to test the hypothesis that environmental determinants of human infection also promote infections in other mammalian hosts.

**Methods**

This study was conducted in three counties in Sichuan Province, China, where schistosomiasis reemerged following the reduction of human and bovine infection prevalence below 1% and the attainment of transmission control according to Chinese Ministry of Health guidelines [6]. In 2007, a cross-sectional survey was conducted in 53 villages. Humans and bovines were tested for *S. japonicum* infection and residents were interviewed to assess characteristics that might promote or impede the distribution of *S. japonicum* eggs, as well as to collect information about potential confounders. The selection of study villages and participation in the parasitological surveys are described in detail in Chapter 3.

**Parasitological surveys.** All residents were asked to complete a brief demographic survey and to submit three stool samples for testing using the miracidial hatch test and the Kato-Katz thick smear procedure [20,21]. One miracidial hatch test was conducted for each stool sample provided. Approximately 30 grams of stool were suspended in aqueous solution, strained with copper mesh to remove large particles, and strained a second time with nylon mesh to concentrate schistosome eggs. The sediment was re-suspended and left in a room where ambient temperatures were between 28 and 30 degrees C. Samples were examined three and five hours after preparation for the presence of miracidia. In addition, three slides were prepared with 41.5 mg each of homogenized stool from the first sample submitted by each participant using the thick smear technique. Slides were examined for *S. japonicum* eggs using a dissecting microscope and if any *S. japonicum* eggs were detected, the species and number of eggs were confirmed by a second reader. A person was classified as infected if any *S. japonicum* miracidia or eggs were detected. Infection intensity, in eggs per gram of stool (EPG), was calculated as the total number of *S. japonicum* eggs present on the slides divided by the total sample weight.

The research team attempted to test all bovines in the study villages for *S. japonicum* infection. Three stool samples were collected from each animal by tying the animal or confining it to a pen until a sample was produced on three separate days. Each sample was tested with the miracidial hatch test using a rapid examination procedure, as the high water content of bovine stool promotes hatching of schistosome eggs and miracidia have short life expectancy in the
media. Samples were examined for miracidia one, two and four hours after preparation. Bovines were classified as infected if any miracidia were observed. Bovines with at least one positive hatch test were subsequently examined using the Danish Bilharziasis Laboratory (DBL) method in order to estimate infection intensity [22]. Five grams of homogenized stool were washed through a series of three sieves (mesh size: 400 µm, 100 µm, 45 µm) and the remaining material was suspended and left in the dark to sediment. The solution was then centrifuged, the top half of the liquid decanted, and the remaining sediment re-suspended and re-centrifuged in order to obtain 2 mL of solution. A thick smear approach was used to count the eggs on up to 30 slides. Infection intensity (EPG) was calculated as the total number of eggs divided by the total sample weight, five grams.

All human and bovine infection surveys were conducted in November and December 2007.

**Assessment of agricultural practices, bovine ownership and sanitation.** In June 2007, the head of each household was asked to complete a structured questionnaire describing household agricultural practices, ownership of domestic animals, socio-economic indicators and sanitation. Participants were asked how many cows and water buffalo were currently owned by members of the household. Participants listed all crops planted in each of the two previous growing seasons: the summer season, typically May to September, and the winter season, typically October to April. For each crop, the participant described the area planted in mu (a commonly used unit by Chinese farmers, equivalent to 667 meters²), the number of times night soil was applied, the amount of night soil applied each time and whether chemical fertilizers were used. Night soil quantities were reported in buckets, as pilot testing indicated standard sized buckets were typically used to transport night soil to fields. The amount of night soil applied by each household was calculated as the total number of buckets of night soil applied to all crops in the past year. Participants also described all latrines in their household. If anaerobic biogas digesters were present, residents were asked if the system was functioning, as pilot testing had indicated some older systems were no longer in use. Households were classified as having improved sanitation if a biogas digester was present and currently used by household members. Any individuals who were tested for *S. japonicum* infection but had no corresponding household or demographic questionnaires were recontacted in spring 2008 to be interviewed.

In light of evidence suggesting *S. japonicum* eggs are removed from the effluent of anaerobic biogas digesters [23], a second biogas-adjusted measure of night soil use was calculated: households with a working anaerobic biogas digester were assigned zero night soil use to account for the inactivation and removal of schistosome eggs; all others were assigned their reported night soil use.

**Ethical approval and treatment.** All participants provided written, informed consent before participating in this study. The research protocol was approved by the Sichuan Institutional Review Board, the National Institutes of Health Institutional Review Board and the University of California, Berkeley, Committee for the Protection of Human Subjects. Every person who tested positive for *S. japonicum* was provided treatment with praziquantel by the county Anti-Schistosomiasis Control Station. All bovines testing positive were referred to the county Department of Animal Husbandry for treatment with praziquantel.

**Estimation of village-level exposures.** Village-level exposure measures were calculated by aggregating household exposure measures for every household in the village, excluding the index household. Regression equations that include both a household-level measure (xₕ) and a village-level measure that summarizes all households in a village (xᵥ) are problematic. Each
parameter in a regression model estimates the change in an outcome, \( y \), based on a unit change in \( x \), holding constant all other variables. But changing \( x_h \) necessarily changes \( x_v \), a concern raised by Oakes in his discussion of the challenges of estimating the impact of neighborhood characteristics on health outcomes [24]. Excluding the index household from the calculation of the ecological variable allows one to consider the theoretical impact of changing village exposures on the outcome, \( y \), while holding constant household exposure, and vice versa. Village-level measures that exclude the index household are denoted here as \( x_{v-h} \). Village-level night soil use was calculated for each household as the mean amount of night soil used by all other households in the village:

\[
x_{v-h} = \frac{\sum_{n=1}^{n} x_n}{n-1} - x_h
\]

Equation 1

where \( n \) is the number of households in the village and \( x_h \) is the volume of night soil applied by a household in the past year. The same equation was used to calculate village measures of bovine density (\( x_h \) = the number of bovines owned by a household), infected bovine density (\( x_h \) = the number of infected bovines owned by a household) and the percent of households with biogas (\( x_h \) = 1 if a biogas digester was currently in use).

**Statistical analysis.** Before examining exposure-disease relationships, the distributions of all exposures were examined for extreme values and the correlations between variables were examined to check for colinearity. Spearman’s rho was used for non-parametrically distributed variables. One outlier was detected: a household reported applying 12,000 buckets of night soil to a single summer crop, a value over 50 times larger than the 99\(^{th}\) percentile value. As the area planted by the household was not unusually large and the estimate had the potential to be influential, night soil values for this household were excluded from the analysis.

Two methods were used to estimate the impact of household- and village-level improved sanitation, night soil use, bovine ownership and bovine infections on human *S. japonicum* infection. First, the impact of each exposure on schistosomiasis was estimated using the following equation:

\[
\logit \left( \Pr(Y_{ibv} = 1 \mid x_h, x_{v-h}, W) = \beta_1 + \beta_2 x_h + \beta_3 x_{v-h} + \beta_4 x_h x_{v-h} + \beta_5 W \right)
\]

Equation 2

where \( Y_{ibv} \) is individual \( i \)’s infection status, and \( W \) is a vector of potential confounders. \( \beta_2 \) estimates the association between household exposure and infection status, controlling for village-level exposures and other confounders. \( \beta_3 \) estimates the impact of village exposure on infection status adjusting for household exposure and other confounders. Both \( \beta_2 \) and \( \beta_3 \) are calculated as a log odds ratio for a one-unit change in household (\( \beta_2 \)) or village (\( \beta_3 \)) exposure. Because it is possible that the impact of village-level exposures on infection risk may differ for those exposed vs. unexposed at the household-level, interactions between household- and village-level exposures were examined and the interaction term included if \( p<0.05 \). Note that the presence of interactions changes the interpretations of \( \beta_2 \) and \( \beta_3 \), as the association between, for example, household exposure and infection status, depends on the level of village exposure and vice versa. Household-level exposures were modeled as binary variables. Village-level exposures were modeled as continuous variables. Village night soil use was scaled such that one
unit is 100 buckets, village biogas coverage was scaled such that one unit is 10% and infected bovine density was scaled such that one unit is 0.1 infected bovines per household. To account for non-linear exposure response relationships, village exposures were also categorized into quartiles. Because quartiles were defined at the household-level, the number of humans or bovines in each quartile may not be exactly 25% of the population. To ensure the comparability of estimates of the impact of night soil and biogas-adjusted night soil use on *S. japonicum* infection, the same quartiles were used for each measure, based on those developed for night soil use. Generalized estimating equations (GEE) using exchangeable correlation structures were used to account for village-level correlation and derive robust inference [25].

Confounders were defined for each exposure based on their potential to influence the distribution of the exposure of interest and independently affect *S. japonicum* infection status [26]. County, SES and the amount of land cultivated were considered possible confounders for all four exposures of interest. These variables likely affect infection status through access to treatment (county, SES) and water contact (SES, cultivated land) and may also impact the distribution of domestic animals, sanitation and night soil use at the household- and village-level. SES was assessed using an asset-based approach given the difficulty of estimating household incomes in agrarian villages [27]. The head of household was asked if any member of the household owned eight different items (a car, tractor, motorcycle, computer, television, washing machine, air conditioner and refrigerator). Households were assigned an inventory score based on the number of items owned. Village-level SES was calculated as the mean score of all households in each village, excluding the index household. For each household, cultivated land area was measured as the total area planted by members of the household in the past year. At the village-level, the mean area cultivated per household, excluding the index household, was calculated. Because measures of the area of land cultivated at the household and village level were right-skewed, the measures were natural log transformed, using an ln(x+1) transformation for the household measure as some families reported not planting any crops in the past year. Due to the strong association observed between age and human infection status (described in Chapter 3), age was considered a potential confounder and modeled as a linear variable. While age may not influence household exposures directly, differences in age distribution between villages may lead to empirical confounding.

The density of bovines, the distribution of biogas and night soil use were also considered potential confounders for each of the exposures of interest. Because each of these variables could cause bovine as well as human infections, infected bovine density was considered a potential effect of each exposure and therefore not included as a potential confounder in models of human infection status.

Second, a parameter akin to attributable risk was estimated in order to compare the contribution of the exposures examined here to human *S. japonicum* infection. Using the population intervention model proposed by Hubbard and Van der Laan [28], the change in the prevalence of human infections was estimated for theoretical interventions that reduced $x_h$ and $x_{(v-h)}$ to zero or, in the case of anaerobic biogas digesters, interventions that increased $x_h$ to 1 and $x_{(v-h)}$ to 100%. This model derives from the Rubin causal model which defines causal parameters in terms of counterfactual outcomes: the impact of exposure A is the difference in outcome Y when an individual is exposed verses unexposed (or exposed at any two distinct exposure levels) under identical circumstances [29]. As only one such exposure scenario is observable for each individual, imputation is required. Here, g-computation was used to estimate human infection prevalence when exposure was eliminated (or in the case of biogas, increased to 100%) and this
estimate was compared to the observed infection prevalence [30,31]. A GEE logistic regression model was fit to estimate the coefficients of the model described in Equation 2, including the interaction term if p<0.05 and accounting for village-level clustering using exchangeable correlation.  

\[ E \left\{ \Pr(Y = 1 \mid x_h = x_h, x_{(v-h)} = x_{(v-h)}), W \right\} \], was estimated for each individual, setting x_h and x_{(v-h)} to zero (or in the case of biogas: 1 and 100%, respectively). This is an estimate of the counterfactual distribution under the following three assumptions: the model is correctly specified, there is no unmeasured confounding and, for any group of individuals defined by the model, the assignment of the exposures of interest (x_h and x_{(v-h)}) are not deterministic (known as the Experimental Treatment Assignment assumption). The parameter estimated was the estimated change in infection prevalence, expressed as a ratio of the observed infection prevalence, 

\[ \frac{\Pr(Y_0 = 1) - \Pr(Y = 1)}{\Pr(Y_0 = 1)} \], where Y_0 is infection status under the theoretical intervention and Y is the observed infection status. Inference for the estimated prevalence for each intervention was derived using bootstrapping [32]. The population was sampled with replacement, using villages as the sampling unit to account for village-level correlation and the original village-based selection process, to obtain a 53 village sample of the original population. The regression model was re-fit to the sampled dataset and prevalence was estimated as above. The procedure was repeated 1,000 times and the 95% confidence interval was estimated from the 2.5th and 97.5th percentile values of the bootstrapped estimates.

As estimation using g-computation is sensitive to the model specified, the population intervention parameters were also estimated using machine learning methods for model selection. The Deletion/Substitution/Addition (D/S/A) algorithm was used to specify the logistic regression model used to estimate population prevalence when exposures were reduced to zero (or in the case of biogas, increased to 100% coverage) [33]. The D/S/A uses a loss-based estimation procedure with 5-fold cross validation to obtain the best fit of the specified covariates. For each exposure of interest, x_h and x_{(v-h)} were forced into the model and all confounders (county, age, and household and village measures of SES, land area cultivated, night soil use, biogas and bovine ownership) were entered as potential covariates. The variable selection procedure allowed two-way interactions, quadratic terms and up to 12 covariates. In addition, the D/S/A was used to specify the logistic regression model for each of the 1,000 sampled populations during the bootstrapping procedure.

Improved sanitation, night soil use and bovine ownership were also examined as predictors of bovine S. japonicum infection in order to compare the environmental determinants of infection in two mammalian hosts. Household and village biogas access and night soil use were examined as described in Equation 2, where Y_{ihv} is the infection status of bovine i. Bovine density was defined only at the village-level as all households where bovines were tested owned bovines. Recognizing potential biological differences between cows and water buffalo, bovine type was examined as a potential effect modifier of the exposures of interest. As with the human infection analyses, county, SES and cultivated land area were considered potential confounders as were the other exposures of interest. The type of bovine (water buffalo or cow) was also considered a potential confounder since biological differences between the two species may affect their infection risk, and the type of bovines households raise may be linked to agricultural practices and the exposures of interest.

In order to better understand patterns of fertilization with human waste, I examined whether night soil use varies by season, crop-type, the use of chemical fertilizers or household...
SES. Both the use of any night soil, a binary variable, and the quantity of night soil applied, in buckets per m² of land cultivated, were examined. Logistic regression was used to test whether the use of night soil varied by each of the four predictors, accounting for correlations within villages using GEE with exchangeable correlation. Because the quantity of night soil applied was highly right-skewed, it was described using four ordered categories representing approximate quartiles (0 buckets per m², 0.1 – 17.0 buckets per m², 17.1 – 39.0 buckets per m² and >39.0 buckets per m²). Ordinal logistic regression was used to examine the association between each of the four predictors and night soil quantity, using a sandwich type estimator for inference accounting for within village residual correlation [34]. As ordinal logistic regression assumes the effect of a predictor on an outcome is constant for each stepwise increase in the outcome, the Brant test was used to check that this parallel regression assumption was not violated.

Tests of statistical significance were conducted setting $\alpha = 0.05$. Data analysis was conducted using Stata, version 10 (StataCorp, College Station, TX, USA) and R, version 2.9.2 (www.r-project.org).

Results

A total of 4,225 people in the 53 study villages completed demographic interviews and 3,009 were tested for *S. japonicum* infection. In addition, 1,784 heads of household were interviewed, including 1,700 (95.3%) interviewed in June 2007 and 84 (4.7%) interviewed in spring 2008. The true population in the 53 study villages and therefore the percentage of individuals or households participating in this study is unknown as official population registers included many people who had left their villages to find work elsewhere. Village leaders indicated almost all households whose residents spent most of their time in the village were included in the household surveys. The study population presented here includes the 2,833 individuals (94.2% of those tested for infection) with a corresponding household interview and complete information on night soil use, sanitation, bovine ownership, SES, area of land cultivated, county of residence, age and infection status. The study population includes an average of 1.9 people per household (range: 1 to 5).

*S. japonicum* infections were detected in 187 individuals (6.6%) in 35 villages. Mean infection intensity was 1.6 EPG.

Most households engaged in year-round farming and had modest socio-economic resources (Table 1). Household assets were limited: 62.4% of participants lived in households with less than three of the eight items on the household inventory. Almost every participant (96.7%) lived in a household that planted crops in the year prior to the survey. The average household cultivated 3,489 m² of land in the past year (range 0 – 30,000 m²) including 1,819 m² during the summer growing season (May through September) and 1,674 m² during the winter growing season (October through April). The area each household planted in the summer was strongly correlated with the area planted in the winter (Spearman’s rho: 0.877).

**Determinants of human *S. japonicum* infection: household sanitation.** Latrines were present in almost every household: 98.1% of participants had a latrine in their home. In 27 villages, every head of household reported having a latrine, and in 50 villages, at least 90% of households had a latrine. Because of this, there was insufficient heterogeneity of exposure to examine village-level sanitation access as a predictor of schistosomiasis. In villages with incomplete sanitation coverage, people with latrines in their home were three times less likely to
Table 1. Description of the study population and the distribution of human *S. japonicum* infections by county, age, SES and agricultural intensity.

<table>
<thead>
<tr>
<th></th>
<th>No.</th>
<th>(%)</th>
<th>% Inf.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>County</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>County 1</td>
<td>1,130</td>
<td>(40)</td>
<td>9.20</td>
</tr>
<tr>
<td>County 2</td>
<td>844</td>
<td>(30)</td>
<td>7.23</td>
</tr>
<tr>
<td>County 3</td>
<td>859</td>
<td>(30)</td>
<td>2.56</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;12</td>
<td>216</td>
<td>(8)</td>
<td>2.78</td>
</tr>
<tr>
<td>12 - 17</td>
<td>119</td>
<td>(4)</td>
<td>5.04</td>
</tr>
<tr>
<td>18 - 29</td>
<td>174</td>
<td>(6)</td>
<td>4.60</td>
</tr>
<tr>
<td>30 - 39</td>
<td>564</td>
<td>(20)</td>
<td>6.56</td>
</tr>
<tr>
<td>40 - 49</td>
<td>647</td>
<td>(23)</td>
<td>7.11</td>
</tr>
<tr>
<td>50+</td>
<td>1,113</td>
<td>(39)</td>
<td>7.55</td>
</tr>
<tr>
<td><strong>Household asset index</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 items owned</td>
<td>91</td>
<td>(3)</td>
<td>6.59</td>
</tr>
<tr>
<td>1 item owned</td>
<td>775</td>
<td>(27)</td>
<td>8.26</td>
</tr>
<tr>
<td>2 items owned</td>
<td>901</td>
<td>(32)</td>
<td>6.99</td>
</tr>
<tr>
<td>3 items owned</td>
<td>697</td>
<td>(25)</td>
<td>5.16</td>
</tr>
<tr>
<td>4+ items owned</td>
<td>369</td>
<td>(13)</td>
<td>4.88</td>
</tr>
<tr>
<td><strong>Village asset index</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.9 - 1.4 items per household</td>
<td>564</td>
<td>(20)</td>
<td>12.06</td>
</tr>
<tr>
<td>1.5 - 1.9 items per household</td>
<td>368</td>
<td>(13)</td>
<td>6.79</td>
</tr>
<tr>
<td>2.0 - 2.4 items per household</td>
<td>1,233</td>
<td>(44)</td>
<td>6.00</td>
</tr>
<tr>
<td>2.5 - 3.0 items per household</td>
<td>668</td>
<td>(24)</td>
<td>2.99</td>
</tr>
<tr>
<td><strong>Household cultivated land area†</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>94</td>
<td>(3)</td>
<td>0.00</td>
</tr>
<tr>
<td>1 - 1,999 m²</td>
<td>561</td>
<td>(20)</td>
<td>6.42</td>
</tr>
<tr>
<td>2,000 – 3,999 m²</td>
<td>1,115</td>
<td>(39)</td>
<td>6.82</td>
</tr>
<tr>
<td>4,000 – 5,999 m²</td>
<td>637</td>
<td>(22)</td>
<td>6.59</td>
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<tr>
<td>6,000 – 28,667 m²</td>
<td>426</td>
<td>(15)</td>
<td>7.75</td>
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<tr>
<td><strong>Village cultivated land area‡</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,463 - 1,999 m² per household</td>
<td>579</td>
<td>(20)</td>
<td>5.18</td>
</tr>
<tr>
<td>2,000 - 3,999 m² per household</td>
<td>1,261</td>
<td>(45)</td>
<td>7.85</td>
</tr>
<tr>
<td>4,000 - 5,999 m² per household</td>
<td>889</td>
<td>(31)</td>
<td>6.41</td>
</tr>
<tr>
<td>6,000 - 9,542 m² per household</td>
<td>104</td>
<td>(4)</td>
<td>0.96</td>
</tr>
</tbody>
</table>

*Mean asset score of all households in a village except the index household.
†Area of land cultivated in the past year, including summer and winter growing seasons.
‡Mean area of land cultivated in the last year by all households in the village, excluding the index household.
Table 2. The association between household use of anaerobic biogas digesters and human *S. japonicum* infection at different levels of village biogas coverage.

<table>
<thead>
<tr>
<th>No biogas in other households</th>
<th>Unadjusted</th>
<th>Adjusted*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Household biogas use</td>
<td>No</td>
<td>922</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>8</td>
</tr>
<tr>
<td>Biogas in 1.6 - 12.0% of other households</td>
<td>Household biogas use</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>93</td>
</tr>
<tr>
<td>Biogas in 12.1 - 24.0% of other households</td>
<td>Household biogas use</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>76</td>
</tr>
<tr>
<td>Biogas in 24.1 - 66.7% of other households</td>
<td>Household biogas use</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>334</td>
</tr>
</tbody>
</table>

*Adjusted for county, age and household and village SES, cultivated land area, night soil use and bovine ownership.

Household biogas ownership and the prevalence of biogas digesters in a village interacted significantly (p=0.043).

---

be infected as those living in houses without latrines (infection prevalence 4.1% vs. 12.7%), a difference that remained significant after controlling for county, age, and village and household SES, cultivated land area, night soil use and bovine ownership (adjusted OR 0.32, 95% CI 0.16 – 0.66).

Biogas digesters were in use in 282 households and present, but not used, in 57 households. The unused systems were typically older as 60% were built at least ten years before the survey. In contrast, 46% of the systems currently in use were built within two years of the survey and 75% built within the last ten years.

No protective effect of biogas digesters was observed and, in some situations, the presence of biogas digesters in the home was associated with an increased risk of human *S. japonicum* infection (Table 2). The relationship between household biogas use and *S. japonicum* infection varied based on the percent of households in the village with biogas (interaction term p=0.043). People with biogas digesters in their household were significantly more likely to be infected than those without biogas if they lived in a village where few other households had biogas. The risk of infection was highest among people who lived in a household with the only biogas digester in the village. In villages where more households owned biogas systems, biogas use was not associated with *S. japonicum* infection.

**Determinants of human *S. japonicum* infection: night soil.** Human waste was commonly used to fertilize crops: 61.0% of participants lived in households where night soil was used at least once in the year prior to the survey and the practice was reported in all 53 villages. Night soil use occurred during both growing seasons, used by 49.7% of households in the
Table 3. The association between household and village night soil use and human *S. japonicum* infection.

<table>
<thead>
<tr>
<th></th>
<th>No. % Inf.</th>
<th>Unadjusted OR (95% CI)</th>
<th>Adjusted* OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. Night soil (quartiles of village use)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Household night soil use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1,104</td>
<td>6.07 1.00 1.00</td>
<td>1.00 0.93 (0.68 - 1.27)</td>
</tr>
<tr>
<td>Yes</td>
<td>1,729</td>
<td>6.94 1.02 (0.78 - 1.34)</td>
<td>0.93 (0.68 - 1.27)</td>
</tr>
<tr>
<td>Village night soil use†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.6 - 25.7 buckets per household</td>
<td>758</td>
<td>4.62 1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>25.8 - 53.0 buckets per household</td>
<td>702</td>
<td>6.27 0.89 (0.42 - 1.90)</td>
<td>1.40 (0.75 - 2.63)</td>
</tr>
<tr>
<td>53.1 - 78.6 buckets per household</td>
<td>711</td>
<td>6.05 1.01 (0.49 - 2.09)</td>
<td>0.99 (0.49 - 2.01)</td>
</tr>
<tr>
<td>78.7 - 244.5 buckets per household</td>
<td>662</td>
<td>9.82 1.46 (0.59 - 3.63)</td>
<td>3.68 (1.55 - 8.76)</td>
</tr>
<tr>
<td><strong>2. Night soil (continuous measure of village use)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Household night soil use (0/1)</td>
<td></td>
<td>1.02 (0.77 - 1.35)</td>
<td>0.93 (0.69 - 1.25)</td>
</tr>
<tr>
<td>Village night soil use†</td>
<td></td>
<td>1.73 (0.94 - 3.19)</td>
<td>2.35 (1.29 - 4.29)</td>
</tr>
<tr>
<td><strong>3. Night soil (quartiles of household and village use)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Household night soil use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>1,104</td>
<td>6.07 1.00 1.00</td>
<td>1.00 0.95 (0.57 - 1.60)</td>
</tr>
<tr>
<td>2 - 40 buckets</td>
<td>600</td>
<td>4.83 0.86 (0.59 - 1.24)</td>
<td>0.82 (0.53 - 1.26)</td>
</tr>
<tr>
<td>41 - 104 buckets</td>
<td>555</td>
<td>7.21 1.10 (0.75 - 1.60)</td>
<td>1.02 (0.69 - 1.52)</td>
</tr>
<tr>
<td>105 - 1570 buckets</td>
<td>574</td>
<td>8.89 1.12 (0.73 - 1.72)</td>
<td>0.95 (0.57 - 1.60)</td>
</tr>
<tr>
<td>Village night soil use†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.6 - 25.7 buckets per household</td>
<td>758</td>
<td>4.62 1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>25.8 - 53.0 buckets per household</td>
<td>702</td>
<td>6.27 0.87 (0.41 - 1.84)</td>
<td>1.37 (0.73 - 2.57)</td>
</tr>
<tr>
<td>53.1 - 78.6 buckets per household</td>
<td>711</td>
<td>6.05 1.00 (0.49 - 2.05)</td>
<td>1.00 (0.50 - 2.01)</td>
</tr>
<tr>
<td>78.7 - 244.5 buckets per household</td>
<td>662</td>
<td>9.82 1.44 (0.58 - 3.54)</td>
<td>3.64 (1.52 - 8.73)</td>
</tr>
<tr>
<td><strong>4. Night soil (continuous measures of household and village use)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Household night soil volume‡</td>
<td></td>
<td>1.03 (0.96 - 1.09)</td>
<td>1.00 (0.93 - 1.07)</td>
</tr>
<tr>
<td>Village night soil use†</td>
<td></td>
<td>1.71 (0.93 - 3.15)</td>
<td>2.34 (1.28 - 4.27)</td>
</tr>
<tr>
<td><strong>5. Night soil from non-biogas sources (quartiles of village use)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-biogas household night soil use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1,420</td>
<td>7.11 1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Yes</td>
<td>1,413</td>
<td>6.09 0.88 (0.69 - 1.11)</td>
<td>0.76 (0.58 - 0.98)</td>
</tr>
<tr>
<td>Non-biogas village night soil use†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.9 - 25.7 buckets per household</td>
<td>1,145</td>
<td>6.11 1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>25.8 - 53.0 buckets per household</td>
<td>742</td>
<td>5.80 0.88 (0.51 - 1.52)</td>
<td>0.77 (0.47 - 1.26)</td>
</tr>
<tr>
<td>53.1 - 78.6 buckets per household</td>
<td>425</td>
<td>3.53 0.88 (0.40 - 1.95)</td>
<td>0.84 (0.45 - 1.56)</td>
</tr>
<tr>
<td>78.7 - 243.7 buckets per household</td>
<td>521</td>
<td>11.32 1.65 (0.73 - 3.73)</td>
<td>1.64 (0.89 - 3.04)</td>
</tr>
<tr>
<td><strong>6. Night soil from non-biogas sources (continuous measures of village use)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-biogas household night soil use (0/1)</td>
<td></td>
<td>0.88 (0.70 - 1.11)</td>
<td>0.77 (0.60 - 1.00)</td>
</tr>
<tr>
<td>Non-biogas village night soil use†</td>
<td></td>
<td>1.28 (0.67 - 2.44)</td>
<td>0.98 (0.47 - 2.04)</td>
</tr>
</tbody>
</table>

*Adjusted for county, age and household and village SES, cultivated land area, bovine ownership and, for models 1 through 4, biogas use.
†Mean night soil use by households in a village in the past year, excluding the index household, scaled such that one unit is equivalent to 100 buckets.
‡Natural log transformed using ln(x+1).
summer and 45.5% in the winter, and the quantity of night soil use used by households in summer vs. winter was correlated (Spearman’s rho=0.689). At the village-level, average household night soil use varied considerably, ranging from four to 245 buckets per household in the past year.

Household night soil use was not associated with human *S. japonicum* infection but night soil use by other community members was (Table 3). Each 100 bucket increase in village night soil use was associated with an approximate doubling of an individual’s infection risk, controlling for household night soil use and all other potential confounders (OR 2.35, 95% CI 1.29 – 4.29). When the quantity of night soil used by household members was substituted for the dichotomous household variable, results were unchanged: village, but not household-level night soil use, was associated with *S. japonicum* infection. No interaction between household and village biogas-adjusted night soil use was observed (p=0.405).

When measures of night soil use excluded night soil use by households that had biogas digesters, which may inactivate and remove *S. japonicum* eggs, no association was observed between schistosomiasis infection and village-level estimates of this biogas-adjusted night soil measure. Household-level night soil use by non-biogas users was associated with a reduced risk of *S. japonicum* infection (adjusted OR 0.77, 95% CI 0.60 – 1.00), a finding of marginal statistical significance.

**Determinants of human *S. japonicum* infection: bovines.** Bovines were present in 50 of the 53 study villages and 37.1% of participants lived in a household with at least one bovine. Cows were more common than water buffalo, owned by 73.9% and 30.2% of bovine owners, respectively. Most bovine owners had only one animal (77.1%), 20.6% owned two bovines and 2.3% owned three or more. At the village level, the average number of bovines per household ranged from 0 to 1.2.
Household bovine ownership and village bovine density were associated with an increased risk of *S. japonicum* infection (Table 4). Infection prevalence was 10.7% among bovine owners and 4.2% among non-owners, suggesting bovine ownership increases the risk of infection. However, adjusting for potential confounders attenuated this relationship (OR 1.33, 95% CI 0.90 – 1.95). At the village-level, the average number of bovines per household was associated with an increased risk of *S. japonicum* infection. Individuals in villages where bovine density was in the upper three quartiles were significantly more likely to be infected than individuals with the lowest quartile of bovine exposure. However, when bovine density was modeled as a continuous variable, this relationship was not statistically significant, suggesting a non-linear exposure-response relationship as shown in Figure 1. There was no interaction between bovine ownership in the household and the density of bovines in a village (p=0.092).

**Determinants of human *S. japonicum* infection: bovine *S. japonicum* infections.** Research teams identified 821 bovines in 50 villages in the fall of 2007 and 537 bovines in 44 villages were tested for infection. Bovine infection prevalence was 13.4%, more than double human infection prevalence, although infection intensity was lower (0.015 EPG). Infected bovines were present in 23 villages. The presence of infected bovines in a household or the density of infected bovines in a village could not be determined for 577 people who lived in households or villages where bovines were present but none were tested. Because of this, tests of the association between bovine *S. japonicum* infections and human schistosomiasis were limited to 2,256 individuals in 47 villages.

**Figure 1.** Lowess smoothed plot of village bovine density as a predictor of *S. japonicum* infection (top), and a histogram of village bovine density among the 2,833 participants (bottom).
Table 5. The relationship between the density of bovine *S. japonicum* infections in a village and human *S. japonicum* infection status in households with and without infected bovines.

<table>
<thead>
<tr>
<th>1. Bovine infections (quartiles)</th>
<th>Unadjusted</th>
<th>Adjusted*</th>
</tr>
</thead>
<tbody>
<tr>
<td>No infected bovines in the household</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Village bovine infection density†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No infected bovines in other households</td>
<td>1,190</td>
<td>4.03</td>
</tr>
<tr>
<td>0.01 - 0.02 infected bovines per household</td>
<td>251</td>
<td>9.96</td>
</tr>
<tr>
<td>0.03 - 0.08 infected bovines per household</td>
<td>408</td>
<td>8.58</td>
</tr>
<tr>
<td>0.09 - 0.49 infected bovines per household</td>
<td>291</td>
<td>11.68</td>
</tr>
<tr>
<td>Infected bovine(s) in the household</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Village bovine infection density‡‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No infected bovines in other households</td>
<td>20</td>
<td>0.00</td>
</tr>
<tr>
<td>0.01 - 0.02 infected bovines per household</td>
<td>5</td>
<td>40.00</td>
</tr>
<tr>
<td>0.03 - 0.08 infected bovines per household</td>
<td>30</td>
<td>33.33</td>
</tr>
<tr>
<td>0.09 - 0.49 infected bovines per household</td>
<td>61</td>
<td>21.31</td>
</tr>
</tbody>
</table>

| 2. Bovine infections (continuous) | | |
| No infected bovines in the household | | |
| Village bovine infection density† | 1.60 (1.23 - 2.07) | 1.98 (1.60 - 2.43) |
| Infected bovine(s) in the household | | |
| Village bovine infection density† | 1.20 (0.90 - 1.59) | 1.33 (1.02 - 1.73) |

| 3. Bovine infections in villages where >50% of bovines tested (continuous)** | | |
| No infected bovines in the household | | |
| Village bovine infection density† | 1.56 (1.18 - 2.06) | 1.98 (1.58 - 2.47) |
| Infected bovine(s) in the household | | |
| Village bovine infection density† | 1.14 (0.84 - 1.56) | 1.32 (1.00 - 1.75) |

*Adjusted for age, county and household and village SES, cultivated land area, night soil use and biogas digester use.
†Mean number of bovines in all households in a village, excluding the index household, scaled such that one unit is a change in 0.1 infected bovines per household.
‡‡Model could not be fit because one group had no infections.
**Includes 1866 participants from 40 villages.
A significant interaction was detected between household ownership of infected bovines and village-level bovine infection density (p=0.006).

Infected bovines were present in the homes of 116 participants (5.1%), including 110 participants with one infected bovine and six participants with two infected bovines. At the village-level, the average number of infected bovines per household ranged from 0 to 0.49.

Participants faced an increased risk of infection if they lived in villages with a greater density of infected bovines (Table 5). The magnitude of this risk depended on the presence of infected bovines in the household (interaction term p=0.006). In households without infected bovines, each additional infected bovine per ten households (a 0.1 unit increase in infected bovine density) was associated with an approximate doubling of infection risk, adjusting for county, age and household and village SES, cultivated land area, night soil use and biogas digester use.
The interaction between household-level ownership of an infected bovine and village-level infected bovine density in predicting human *S. japonicum* infection. Odds ratios (dots) and 95% confidence intervals (lines) for *S. japonicum* infection among people with vs. without infected bovines in their household at different densities of infected bovines at the village-level are shown.

Ownership (OR 1.98, 95% CI 1.60 – 2.43). In households with infected bovines, the impact of infected bovine density in the village on infection risk was smaller, adjusting for the same confounders (OR 1.33, 95% CI 1.02 – 1.73). Sensitivity analyses that limited the sample to the 40 villages where at least 50% of bovines were tested for infection (or no bovines were present) yielded minimal changes in estimates of the relationship between infected bovine density and human schistosomiasis prevalence.

Similarly, in villages with a low density of infected bovines, the odds of infection were higher among participants who owned infected bovines compared to those without infected bovines (Figure 2). In villages with higher densities of infected bovines, household bovine ownership was not associated with an increased risk of infection, demonstrating that the importance of household exposures may vary based on the level of village exposures and vice versa.

**Comparison of the impact of sanitation, night soil use and bovines on human *S. japonicum* infection.** Table 6 shows the estimated change in human *S. japonicum* infection prevalence following the implementation of five theoretical interventions to reduce *S. japonicum* egg dispersal. The removal of all bovines yielded the largest estimated reductions in infection prevalence, reducing infections by 52% using fixed model estimation or by 72% using machine learning procedures. Eliminating bovine infections yielded smaller estimated reductions in infection. Ending the practice of night soil use yielded the second largest reduction in infection prevalence, a 37% reduction using fixed model estimation. Machine learning estimates were
Table 6. Estimated change in human *S. japonicum* infection prevalence following interventions to reduce egg dispersal using two different estimation procedures. The estimated change in human infection prevalence is presented as a fraction of the observed infection prevalence, 6.60%.

<table>
<thead>
<tr>
<th>Theoretical intervention</th>
<th>N</th>
<th>Change in prevalence (95% CI) estimated with a fixed model*†</th>
<th>Change in prevalence (95% CI) estimated with machine learning*‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Install biogas in all households</td>
<td>2,833</td>
<td>1.27 (-0.78 - 6.06)</td>
<td>2.06 (-1 - 14.12)</td>
</tr>
<tr>
<td>Eliminate night soil use</td>
<td>2,833</td>
<td>-0.37 (-0.68 - 0.1)</td>
<td>-0.35 (-0.86 - 0.75)</td>
</tr>
<tr>
<td>Eliminate night soil use in households without biogas</td>
<td>2,833</td>
<td>0.15 (-0.4 - 0.91)</td>
<td>-0.15 (-0.79 - 1.95)</td>
</tr>
<tr>
<td>Remove all bovines</td>
<td>2,833</td>
<td>-0.52 (-0.81 - 0.06)</td>
<td>-0.72 (-0.95 - 1.56)</td>
</tr>
<tr>
<td>Eliminate bovine infections</td>
<td>2,256</td>
<td>-0.23 (-0.57 - 0.14)</td>
<td>-0.24 (-0.58 - 0.23)</td>
</tr>
</tbody>
</table>

*Change in prevalence was calculated using the following formula, \( \frac{Pr(Y_0 = 1)}{Pr(Y = 1)} - \frac{Pr(Y = 1)}{Pr(Y_0 = 1)} \), where \( Y_0 \) is infection status under the theoretical intervention and \( Y \) is the observed infection status, 6.60%.
†The impact of each theoretical intervention was estimated using a GEE logistic regression model including household and village exposures, household-village interaction if significant and all potential confounders.
‡The impact of each theoretical intervention was estimated using a machine learning method to select a model, forcing household and village exposures into the model, and allowing for selection of all potential confounders, interactions and quadratic terms.

Confidence intervals were based on 1,000 bootstrapped estimates of infection prevalence. For each estimate, the population was resampled with replacement by village, the model re-selected (machine learning only), re-fit and prevalence estimated based on the theoretical intervention. The 2.5 and 97.5 percentile values are reported.

similar. Biogas installation in all households yielded an estimated infection prevalence above observed values. There is considerable uncertainty around the estimates and all 95% confidence intervals include 1. The uncertainty was greater when machine learning was used for model selection compared to fixed model estimation.

**Determinants of bovine *S. japonicum* infection.** Of the 537 bovines tested for infection, 498 bovines (92.7%) in 43 villages had complete information on county of residence, bovine type, infection status, household night soil use, sanitation, SES and area of land cultivated. This includes 386 cows and 112 water buffalo. *S. japonicum* infections were detected in 66 of the 498 bovines (13.3%). Bovine schistosomiasis was detected in 20 of the 43 villages. Infection prevalence did not vary significantly between cows (14.2%) and water buffalo (9.8%, \( p=0.507 \)).

The density of bovines in a village was a strong and significant predictor of bovine infection status (Table 7). An increase in one bovine per household increased the odds of infection by a factor of 14.7, but there was considerable uncertainty around this estimate (95% CI 3.63 – 59.38). Bovine infection prevalence also increased with village night soil use but this relationship was dampened in models that adjusted for potential confounders. When night soil use from only non-biogas sources was considered, bovines in the upper two quartiles of exposure were significantly more likely to be infected than those in the lowest exposure quartile, adjusting for potential confounders. However, this relationship was distinctly non-linear, as the odds of infection in the third exposure quartile were greater than the odds of infection in the fourth exposure quartile. Direct measures of household and village improved sanitation were not
Table 7. The associations between improved sanitation, night soil use, bovine density and bovine *S. japonicum* infection.

<table>
<thead>
<tr>
<th>1. Biogas (quartiles)</th>
<th>No.</th>
<th>% Inf.</th>
<th>Unadjusted OR (95% CI)</th>
<th>Adjusted* OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Household biogas</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>412</td>
<td>13.3</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Yes</td>
<td>86</td>
<td>12.8</td>
<td>0.73 (0.43 - 1.22)</td>
<td>0.52 (0.28 - 0.98)</td>
</tr>
<tr>
<td>Village biogas coverage†</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No biogas</td>
<td>222</td>
<td>10.8</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>1 - 12.0%</td>
<td>49</td>
<td>12.2</td>
<td>1.33 (0.39 - 4.57)</td>
<td>2.07 (0.40 - 10.80)</td>
</tr>
<tr>
<td>12.1 - 24.0%</td>
<td>110</td>
<td>20.0</td>
<td>1.75 (0.44 - 6.95)</td>
<td>5.05 (0.73 - 34.97)</td>
</tr>
<tr>
<td>24.1 - 66.7%</td>
<td>117</td>
<td>12.0</td>
<td>1.18 (0.37 - 3.74)</td>
<td>0.54 (0.16 - 1.76)</td>
</tr>
<tr>
<td>2. Biogas (continuous)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Household biogas (0/1)</td>
<td></td>
<td></td>
<td>0.70 (0.42 - 1.18)</td>
<td>0.50 (0.26 - 0.98)</td>
</tr>
<tr>
<td>Village biogas coverage†</td>
<td></td>
<td></td>
<td>1.05 (0.86 - 1.27)</td>
<td>0.89 (0.73 - 1.08)</td>
</tr>
<tr>
<td>3. Night soil (quartiles)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Household night soil use</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>142</td>
<td>15.5</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Yes</td>
<td>356</td>
<td>12.4</td>
<td>0.71 (0.37 - 1.35)</td>
<td>0.69 (0.35 - 1.35)</td>
</tr>
<tr>
<td>Village night soil use‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15.3 - 25.7 buckets per household</td>
<td>75</td>
<td>4.0</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>25.8 - 53.0 buckets per household</td>
<td>104</td>
<td>8.7</td>
<td>2.79 (0.63 - 12.35)</td>
<td>2.65 (0.35 - 19.91)</td>
</tr>
<tr>
<td>53.1 - 78.6 buckets per household</td>
<td>133</td>
<td>12.8</td>
<td>5.53 (1.09 - 27.97)</td>
<td>4.40 (0.48 - 40.13)</td>
</tr>
<tr>
<td>78.7 - 243.7 buckets per household</td>
<td>186</td>
<td>19.9</td>
<td>5.23 (0.90 - 30.42)</td>
<td>3.16 (0.31 - 32.54)</td>
</tr>
<tr>
<td>4. Night soil (continuous)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Household night soil use (0/1)</td>
<td></td>
<td></td>
<td>0.71 (0.36 - 1.40)</td>
<td>0.68 (0.35 - 1.33)</td>
</tr>
<tr>
<td>Village night soil use‡</td>
<td></td>
<td></td>
<td>1.54 (0.83 - 2.86)</td>
<td>1.06 (0.54 - 2.06)</td>
</tr>
<tr>
<td>5. Night soil from non-biogas sources (quartiles)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Household night soil use, biogas adjusted</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>201</td>
<td>13.9</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Yes</td>
<td>297</td>
<td>12.8</td>
<td>0.92 (0.56 - 1.54)</td>
<td>1.05 (0.68 - 1.63)</td>
</tr>
<tr>
<td>Village night soil use, biogas adjusted‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.1 - 25.7 buckets per household</td>
<td>145</td>
<td>6.9</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>25.8 - 53.0 buckets per household</td>
<td>127</td>
<td>8.7</td>
<td>1.72 (0.51 - 5.85)</td>
<td>2.42 (0.84 - 6.99)</td>
</tr>
<tr>
<td>53.1 - 78.6 buckets per household</td>
<td>66</td>
<td>22.7</td>
<td>6.22 (1.67 - 23.15)</td>
<td>7.77 (2.25 - 26.85)</td>
</tr>
<tr>
<td>78.7 - 243.7 buckets per household</td>
<td>160</td>
<td>18.8</td>
<td>3.34 (0.91 - 12.22)</td>
<td>3.88 (1.23 - 12.25)</td>
</tr>
<tr>
<td>6. Night soil from non-biogas sources (continuous)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Household night soil use, biogas adjusted (0/1)</td>
<td></td>
<td></td>
<td>1.02 (0.55 - 1.88)</td>
<td>1.15 (0.65 - 2.03)</td>
</tr>
<tr>
<td>Village night soil use, biogas adjusted‡</td>
<td></td>
<td></td>
<td>1.26 (0.70 - 2.26)</td>
<td>1.16 (0.47 - 2.86)</td>
</tr>
</tbody>
</table>

Continued on next page
Table 7. The associations between improved sanitation, night soil use, bovine density and bovine *S. japonicum* infection (continued).

<table>
<thead>
<tr>
<th>7. Bovine density (quartiles)</th>
<th>No.</th>
<th>% Inf.</th>
<th>Unadjusted OR (95% CI)</th>
<th>Adjusted* OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Village bovine density**</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.01 - 0.10 bovines per household</td>
<td>14</td>
<td>7.1</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>0.11 - 0.39 bovines per household</td>
<td>79</td>
<td>8.9</td>
<td>1.33 (0.15 - 12.14)</td>
<td>4.66 (0.51 - 42.84)</td>
</tr>
<tr>
<td>0.40 - 0.76 bovines per household</td>
<td>153</td>
<td>7.2</td>
<td>1.13 (0.16 - 7.81)</td>
<td>5.64 (0.67 - 47.48)</td>
</tr>
<tr>
<td>0.77 - 1.14 bovines per household</td>
<td>252</td>
<td>18.7</td>
<td>3.23 (0.44 - 23.81)</td>
<td>20.31 (2.36 - 174.65)</td>
</tr>
</tbody>
</table>

8. Bovine density (continuous)

<table>
<thead>
<tr>
<th>Village bovine density**</th>
<th>Unadjusted OR (95% CI)</th>
<th>Adjusted* OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5.65 (0.92 - 34.56)</td>
<td>14.69 (3.63 - 59.38)</td>
</tr>
</tbody>
</table>

*Adjusted for county, bovine type, household and village SES and cultivated land area. Each analysis also adjusted for all other predictors: bovine density, household and village biogas use and night soil use.
†Percent of households in a village with biogas, excluding the index household. Scaled such that one unit = 10% biogas coverage.
‡Mean buckets of night soil used per household in a village, excluding the index household. Scaled such that one unit = 100 buckets per household.
**Mean number of bovines per household in a village, excluding the index household.

associated with bovine infection. No significant interactions between household and village exposures or between the exposures and bovine type were detected.

**Night soil fertilization practices.** During the summer growing season, rice and corn accounted for 92.7% of the total cultivated land area. Peanuts and vegetables were also planted in limited quantities. During the winter growing season, rapeseed and wheat covered 97.4% of the total cultivated land area. Night soil was applied to all major crops, typically one to two times during the growing season (Table 8). Night soil fertilization practices varied by crop type: 43.3% of households that grew rice fertilized them with night soil using, on average, 16.2 buckets of night soil per m² of land in the past year. In contrast, night soil was used by 56.6% of corn farmers who applied, on average, 40.4 buckets per m² of land in the past year. Despite these differences, the greatest volume of night soil was applied to the crops that covered the greatest land area (Figure 3). Chemical fertilizers were used by 99.2% of households that reported planting crops in the past year. While the use of night soil varied little by household asset score, the quantity of night soil applied was lower in households with more assets. However, this association was of marginal statistical significance (p=0.053).

Most people used human waste from their own latrines as fertilizer, but there is evidence that night soil trading did occur between households. In 112 households, people reported using human waste from other families as fertilizer and in 137 households, people reported providing night soil to individuals from another household.

**Discussion**

The use of human waste as an agricultural fertilizer and the density of bovines in a village predicted human *S. japonicum* infection, providing evidence that village-level conditions that facilitate the distribution of schistosome eggs from human and other mammalian hosts are important determinants of human schistosomiasis in a region where schistosomiasis reemerged.
The density of bovines in a village, but not night soil use, was associated with bovine *S. japonicum* infection status, suggesting that bovine infections are acquired primarily from eggs from other cows and water buffalo, rather than schistosome eggs from human hosts. The quantity of eggs released by humans and bovines was limited in this region as infection intensities in both hosts were low. The use of night soil and the presence of wandering (and freely defecating) bovines may provide crucial links in the schistosome life cycle, promoting mammalian infections in areas where parasite populations have been reduced.

Household sanitation has long been recognized for its potential to reduce schistosomiasis transmission [35-37]. In this study population, almost every household had a latrine (98%), and latrine ownership was associated with reduced infection prevalence. But the practice of extracting waste from stool pits and spreading it in agricultural areas appears to counter the waste-containing benefits that latrines offer. Night soil was widely used, on all major crops, in both winter and summer planting seasons and by residents in all 53 study villages. Ending this practice could reduce infection prevalence by an estimated 37%, however there is considerable uncertainty around this estimate. Night soil use has largely been overlooked: recent schistosomiasis control strategies in China have emphasized integrated control measures, including removal of bovines and improvements to sanitation, but have not addressed the role of night soil in schistosomiasis transmission [12]. Night soil may also promote the transmission of other fecal-borne pathogens, particularly parasites such as ascaris, that can withstand prolonged environmental exposure [38]. Further research is needed to assess the contribution of night soil to schistosomiasis transmission in other areas, identify other infections that can be transmitted by the use of night soil and to estimate the burden of disease resulting from this practice.

In the study region, night soil is typically a combination of human and pig waste, with pigs contributing most of the volume. Efforts to separate the two sources and use only pig waste as fertilizer may reduce the risks presented by night soil use. Pigs are usually kept in pens constructed so that their waste is captured in the same pit that collects human waste. Using estimates of daily stool output [16], a household with three people and two pigs might find that
Table 8. Night soil use by crop type and SES among the 1,686 households that planted crops in the past year.

<table>
<thead>
<tr>
<th>Plant the following crops in...</th>
<th>No. households</th>
<th>Use night soil (%)</th>
<th>p-value*</th>
<th>Mean volume night soil (buckets per m²)</th>
<th>p-value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grow crops in...</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Summer</td>
<td>1,673</td>
<td>52.90</td>
<td>0.025</td>
<td>21.60</td>
<td>NA‡</td>
</tr>
<tr>
<td>Winter</td>
<td>1,660</td>
<td>48.73</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rice</td>
<td>1,435</td>
<td>43.34</td>
<td>&lt;0.001</td>
<td>16.21</td>
<td>NA‡</td>
</tr>
<tr>
<td>Corn</td>
<td>738</td>
<td>56.64</td>
<td></td>
<td>40.37</td>
<td></td>
</tr>
<tr>
<td>Vegetables</td>
<td>207</td>
<td>65.22</td>
<td></td>
<td>59.20</td>
<td></td>
</tr>
<tr>
<td>Peanuts</td>
<td>217</td>
<td>33.18</td>
<td></td>
<td>14.28</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>34</td>
<td>50.00</td>
<td></td>
<td>79.91</td>
<td></td>
</tr>
<tr>
<td>Plant the following crops in winter</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rapeseed</td>
<td>1,487</td>
<td>47.21</td>
<td>&lt;0.001</td>
<td>25.05</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Wheat</td>
<td>989</td>
<td>34.28</td>
<td></td>
<td>14.88</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>122</td>
<td>66.39</td>
<td></td>
<td>35.98</td>
<td></td>
</tr>
<tr>
<td>Household asset index</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 items owned</td>
<td>72</td>
<td>63.89</td>
<td>0.724</td>
<td>31.57</td>
<td>0.053</td>
</tr>
<tr>
<td>1 item owned</td>
<td>520</td>
<td>65.00</td>
<td></td>
<td>25.17</td>
<td></td>
</tr>
<tr>
<td>2 items owned</td>
<td>531</td>
<td>57.82</td>
<td></td>
<td>20.56</td>
<td></td>
</tr>
<tr>
<td>3 items owned</td>
<td>375</td>
<td>57.87</td>
<td></td>
<td>18.21</td>
<td></td>
</tr>
<tr>
<td>4+ items owned</td>
<td>187</td>
<td>57.22</td>
<td></td>
<td>15.30</td>
<td></td>
</tr>
</tbody>
</table>

*The Wald test with GEE logistic regression, adjusted for within village correlation, was used.
†The Wald test with ordinal logistic regression, adjusting for within village correlation and categorizing mean night soil volume into approximate quartiles, was used.
‡Could not be calculated due to a violation of the parallel regression assumption.

86% of the waste collected is from pigs. Using only pig waste would result in only minimal reductions in the quantity of fertilizer available. Pig waste is not pathogen free and, as pigs are viable *S. japonicum* hosts, may contain schistosome eggs. But the animals have little opportunity for water contact as they are kept penned and infection prevalence is typically low [39,40].

Another alternative to ending the practice of night soil use altogether is the treatment of waste before it is extracted for use as fertilizer to remove or destroy schistosome eggs and other pathogens. Experimental studies have demonstrated anaerobic biogas digesters can destroy *S. japonicum* eggs through chemical inactivation and remove them through sedimentation [23]. Anaerobic processes have also been shown to reduce concentrations of other enteric pathogens [41,42]. However, no protective effect of biogas was observed in this study. In fact, in villages where few biogas digesters were present, individuals living in households with biogas were at increased risk of infection compared to village residents without biogas. Further, measures that included all night soil use were more strongly associated with human *S. japonicum* infection than measures that included night soil use only by households without biogas. This is the opposite of what was expected. If biogas digesters do remove schistosome eggs, measures of night soil use...
that include only households without biogas digesters should provide a more precise estimate of egg dispersal and, due to the reduction in measurement error, be more strongly associated with infection status, if a true relationship exists. There are several possible explanations for these surprising findings. First, it is possible that high-risk individuals for schistosomiasis infection were targeted for or sought out biogas digesters. The construction of these systems has been promoted by rural energy departments through the provision of technical support and subsidies, but evidence that biogas digesters may remove schistosome eggs from effluent has prompted collaboration with county health departments in some areas, potentially leading to the construction of biogas in areas at high risk of schistosomiasis. Second, it is possible that residual confounding may explain the higher prevalence of infection in biogas owners who live in villages where few other biogas systems have been constructed. While analyses controlled for SES and agricultural activities, it is recommended that households with an anaerobic biogas digester own at least two pigs which should contribute waste to the stool pit in order to ensure sufficient methane production. We did not collect information on pig ownership and it is possible families that own pigs may be distinct from the remaining population and face infection risks that cannot be fully accounted for by the confounders included in this analysis. Third, construction of biogas digesters is more expensive and complex than simpler latrines. Because of this, systems may not always be built to standard specifications, leading to less chemical inactivation and sedimentation of schistosome eggs than observed in experimental studies using properly built systems. Further research is needed to clarify the impact of biogas systems on schistosomiasis transmission. Recent findings suggest a modified, two-stage anaerobic biogas design may be more effective at destroying pathogenic bacteria [43]. The impact of such a design on schistosome destruction is worthy of examination. In addition, the experiments conducted by Remais et al. [23] should be repeated in biogas systems in the study region to assess whether biological and chemical inactivation of schistosome eggs is occurring. If so, community randomized trials may reduce confounding and provide a more definitive estimate of the impact of improved sanitation systems on schistosomiasis.

The density of bovines and infected bovines in a village predicted human S. japonicum infection, suggesting these animals play an important role in human schistosomiasis in reemerging areas. Individuals who lived in villages with the greatest density of bovines or S. japonicum-infected bovines faced a seven-fold increase in infection risk. The removal of bovines from the study area yielded the largest theoretical reduction in human infection prevalence, but the confidence interval on this estimate is wide. Due to their large stool output that may occur throughout the village, bovines appear to facilitate the distribution of schistosome eggs in ways that can sustain human infection prevalence, even when infection intensities are low. These findings are consistent with experimental evidence from endemic areas, particularly the lakes and marshland regions of China [17,19], and studies using microsatellites which suggest considerable parasite exchange occurs between humans and bovines [44]. Notably, the estimated reduction in infection prevalence following the removal of all bovines was greater than the reduction estimated if infected bovines were removed or cured (-0.52 vs. -0.23). This finding should be interpreted cautiously. As bovines only release S. japonicum eggs when infected, it is doubtful that uninfected bovines promote human schistosomiasis. The finding does suggest that the cross-sectional measure of bovine infections may not capture the full contribution of schistosome eggs by bovines. Bovine treatment was common, 81% of owners reported their bovines had been treated for schistosomiasis, over 75% within the past two years. It is possible that many cows and water buffalo uninfected at the time of the survey may have been infected at
some point prior. While every effort was made to process bovine samples quickly in order to avoid the hatching of schistosome eggs before the stool could be examined, it is also possible that some infections were missed [45].

Work on a bovine schistosomiasis vaccine is underway [46,47], but currently, aggressive bovine testing and treatment appears necessary, ideally synchronized with human treatment in order to avoid shifting parasites between reservoirs. While praziquantel cures over 90% of bovine infections, reinfection following treatment is common in endemic areas [48]. The fact that 13.4% of bovines were infected despite recent, widespread treatment suggests reinfection rates were high in this study population and treatment may need to occur frequently. The findings also suggest infected bovines present a risk to the entire community. The detection of an infected bovine should be followed by testing of all villagers, not just the bovine owner, for schistosomiasis. In some areas, schistosomiasis control strategies have included the removal of bovines and their replacement with tractors [12]. This practice could remove the infection hazards presented by bovines, but the benefits of this strategy must be carefully weighed against the potential social impacts and financial costs [49]. Anecdotally, many farmers in the region report raising bovines to increase their financial security, selling them when money is needed for school fees or medical expenses. Tractor use may also be difficult on the hilly terrain that covers much of the endemic and controlled regions of Sichuan province.

Bovine schistosomiasis was, like human schistosomiasis, defined by local environments, primarily the density of bovines in a village. Bovines were also more likely to be infected in villages where more night soil was used, but this association was minimal after accounting for confounders. While inference on predictors of bovine infection may have been limited by the number of bovines included in the sample (498 bovines compared to 2,833 people), these findings suggest bovines acquire infections locally, primarily from other bovines. Bovines are used primarily for plowing, which occurs only at certain times in the agricultural calendar. Much of the rest of the time is spent wandering to graze or tethered in the owner’s yard. It is possible cows and water buffalo frequent the same water contact sites where eggs may be deposited and infections acquired. More work is needed to better characterize bovine exposures and risk factors for infection.

This study demonstrated that village-level characteristics can impact an individual’s infection risk, even when controlling for the same characteristics measured at the household-level. While the ecological fallacy cautions against assigning causation based on group-level measures, sometimes group-level measures are not simply a poor proxy for individual-level measures but the exposure of interest [50,51]. This is particularly true for infectious diseases where an individual’s infection risk is often defined by an exposure environment that includes locations shared by other community members and the transmission of infections requires the passage of infections from one individual to another (in the case of schistosomiasis, via a snail). This phenomenon, termed dependant happenings, is at the heart of the methodological challenges facing infectious disease epidemiology [52]. Models of disease causation typically assume independence: that the disease outcome of one individual is independent of the exposure status of others, called the Stable Unit Treatment Value Assignment (SUTVA) assumption [29]. One way to address this challenge is to examine outcomes in individuals who are unexposed at the household level, at different levels of village exposure. This design was recently used in an study of herd immunity: through a randomized community trial, the administration of influenza vaccines to children was shown to lower the risk of infection in unvaccinated community members, including adults [53]. However, this model fails to account for the impact of group-
level exposures on all group members, including those exposed at the individual level. For example, the protective effect of the cholera vaccine has been shown to hinge not only on individual vaccination status but the percent of people in the community that have been vaccinated [54]. An alternative method for accounting for the interdependence of community members is to account for both community and individual-level exposures (or, in this study, household-level exposures) in the same model. One problem that can arise when this method is used is that the individual-level exposure appears twice in the model, once in the individual-level exposure measure and again, as part of the summary group-level measure. This poses theoretical challenges when estimating the impact of changing group-level exposures, holding constant individual-level exposures, and vice versa. Here, endogeneity in the group-level measure was avoided by defining village-level exposure for each individual based on all households in the village, excluding the individual’s own household. This study underscores the importance of the appropriate specification of exposure-measures for future studies of schistosomiasis and other environmentally mediated pathogens. An analysis that examined only household-level bovine ownership and night soil use may have failed to identify these important determinants of infection.

In this analysis, I estimated two parameters in order to evaluate the impact of bovines, night soil use and improved sanitation on reemerging schistosomiasis: the odds ratio and a population intervention estimate akin to an attributable risk. Both methods implicated similar key predictors of infection, but the inference differed. The odds ratios showed bovine density, infected bovine density and night soil use significantly increased human infection risk. The population intervention estimates showed declines in human infection prevalence if each of these exposures were removed, but the 95% confidence intervals included the observed infection prevalence. The parameters are distinct, and therefore any comparison should be made with caution. However, it is worth noting that two different estimation procedures were used to derive inference, the Huber/White/sandwich estimator of variance was used to estimate confidence intervals for odds ratios whereas confidence intervals for the population intervention estimates were estimated using bootstrapping, a replication-based approach. In addition, when the uncertainty of model selection was acknowledged and machine learning was used to select a regression model for each iteration of the bootstrap, the confidence intervals of the population intervention estimates became wider. The population intervention model using a fixed regression model does account for potential model misspecification, but the differences between the uncertainty estimates for the fixed and machine learning results underscore the difficulty of appropriate model selection here and in many regression analyses, leading to biased results. There are likely second-order terms and non-linear relationships behind these differences.

In post-control areas such as the current study region, where human and bovine *S. japonicum* infection intensities are low, the dynamics of transmission may be sensitive to a diverse source of parasite eggs. The findings here demonstrated the important role of *S. japonicum* eggs from local human and bovine stool in sustaining human infections. Other sources of parasites may also contribute to the reemergence of transmission and the rise in infection prevalence following control efforts. Parasites may be imported via hydrological networks or the movements of the primary hosts, a phenomenon that may sustain transmission in environments otherwise poorly suited to transmission [7,55]. Other mammalian hosts may also serve as reservoirs for *S. japonicum* including dogs, cats, goats, pigs and rats. Recent studies in China and the Philippines have found high infection prevalence in rats and dogs [39,40]. The relative importance of each parasite source may hinge on the magnitude of egg inputs from other
sources. In areas where bovines are absent or not infected, molecular evidence suggests parasite exchange between humans and multiple mammalian reservoirs including cats, dogs and rats [56,57]. It is possible that intensive control focused on bovines may increase the importance of other mammalian reservoirs, a possibility worthy of investigation.

The models presented here make several assumptions that should be considered when interpreting the study findings. First, exposures are assumed to precede infection, an assumption that cannot be evaluated when infection prevalence measures are used. Longitudinal studies that assess schistosomiasis incidence can establish temporality and are needed to replicate or refute the findings presented here. Second, all non-independence between participants was assumed to be described by the group-level variables included in the model and villages were assumed to be independent. As discussed above, parasites may cross village boundaries through hydrological and social networks.

In addition, the impact of measurement error, selection bias and confounding on study results should not be overlooked. Infection surveys were designed to maximize sensitivity and minimize measurement error: human *S. japonicum* infection status was assessed using two parasitological methods and three stool samples, bovine infection status was assessed using one parasitological method (given the labor required for the quantitative DBL test, only positive bovines were examined using this second technique), and three stool samples. Nonetheless, egg output by an infected individual may vary considerably and given low infection intensities, it is likely some people and bovines were wrongly classified as uninfected [58]. Self-reported measures of night soil use, bovine ownership and sanitation, as well as SES and cultivated land area, were used rather than objective measures. As participants were interviewed before infection examinations were conducted, measurement error is likely to be non-differential, biasing results to the null and underestimating the true exposure-disease relationships. Due to the low percent of bovines tested in some villages, estimates of infected bovine density may be subject to measurement error. When the sample was limited to villages where at least 50% of bovines were tested, effect estimates and inference were minimally changed, suggesting measurement error is not severely impacting these estimates. Selection bias and participation in the infection surveys is discussed in detail in Chapter 3. Estimates of infection prevalence following theoretic interventions may be affected by the omission of younger, migrant populations, but the results presented here are applicable to those individuals who spent most of their time in the village and are appropriate targets for interventions. I adjusted for a number of potential confounders including SES, cultivated land area, age, county and other exposure measures, but residual confounding may bias effect estimates, especially estimates of the impact of biogas on schistosomiasis infection, as discussed above.

This is one of the first studies to identify high-risk environments for schistosomiasis in a reemerging region. Areas suitable for endemic schistosomiasis have previously been identified based on snail habitat, using remote sensing to identify areas with appropriate temperature, wetness and vegetation [59-61], and the potential for hazardous water contact, primarily through the construction of irrigation systems and dams [62]. The findings here support the findings of mathematical models that conditions that promote the distribution of schistosome eggs facilitate schistosomiasis transmission. The declines in schistosomiasis morbidity and infections due to praziquantel-based control programs are laudable, and have ushered in a new phase of disease control in China and elsewhere. Sustaining reductions in schistosomiasis and even eliminating human infections will require more than chemotherapy. Interventions that impair the distribution of human and bovine schistosome eggs will likely yield further reductions in infections and
prevent reemergence in areas where schistosomiasis has been reduced below detectable levels. The ability to identify high-risk environments for reemergence can aid in focusing surveillance and intervention efforts and promote the long-term control of schistosomiasis.

References


CHAPTER 5

Conclusion

Pharmaceuticals to treat schistosomiasis and other neglected tropical diseases remain unavailable in many endemic regions, despite the fact that these treatments are inexpensive and can yield dramatic declines in morbidity [1,2]. Triage-like morbidity control is urgently required in such areas. But the research presented in the previous chapters demonstrates drug-distribution is only one step in the sustainable control of neglected tropical diseases. The long-term control of schistosomiasis requires, in addition to chemotherapy, reliable infection and morbidity surveillance tools, as well a better understanding of the way in which local environments can promote or impair the parasite life cycle. This information can guide interventions to supplement treatment and focus surveillance efforts. Here, four key findings are discussed, as are their implications for disease control programs, future research and responses to emerging schistosomiasis threats, including climate change and natural disasters.

Key Findings

Widespread reemergence of schistosomiasis has occurred in southwest China five to fifteen years after human infection prevalence was reduced below 1%, underscoring the challenges of attaining permanent schistosomiasis control.

In the absence of a vaccine and in the presence of numerous non-human reservoirs, *S. japonicum* is a poor candidate for eradication [3]. High rates of reinfection following treatment with praziquantel have been observed for all three major schistosome species [4]. This was also observed in the longitudinal analysis described in Chapter 2: two years after all residents who tested positive for *S. japonicum* infection were provided treatment, infection prevalence was 32%. Clearly, reducing schistosomiasis infection levels below a disease control threshold can be challenging. But the findings presented in Chapter 3 suggest that even if disease control criteria are met and sustained for years, the threat of reemergence remains. Limited outbreaks of infections in controlled areas have previously been documented, suggesting episodic renewal of human transmission [5-8]. But I found human infections were widespread – prevalence was 6.5% overall and greater than 20.0% in six villages – suggesting the schistosomiasis transmission cycle had fully reignited in many previously controlled villages. While several countries, including Japan, have successfully eliminated schistosomiasis, reductions in schistosomiasis in many areas may be transient [9,10].

This raises questions about what, exactly, long term schistosomiasis control goals should be. Strict definitions of disease elimination typically involve the reduction of incident infections to zero, but the value of eliminating clinical manifestations of a disease without completely
eliminating incident infections has been recognized [3]. For example, global programs are underway to eliminate blindness resulting from onchocerciasis and trachoma [11-13]. Elimination programs that first target gross morbidity are appropriate when the feasibility of complete elimination is unknown, providing a more attainable and less costly goal [14]. As has been the case with onchocerciasis, such programs may pave the way for later consideration of disease eradication [15,16]. An evaluation conducted from 1989 to 1992 classified schistosomiasis as “not now eradicable” due to the large number of reservoir hosts and snail breeding sites [3]. China is currently challenging that conclusion, attempting to reduce infection prevalence below 1% throughout the world’s most populous country [17]. Prevalence-based targets may be appropriate, but the public health implications of these targets have not been fully evaluated. Morbidity-based targets that focus on the elimination of schistosomiasis-induced anemia, haematuria (in the case of *S. haematobium*) or advanced liver fibrosis (*S. japonicum* and *S. mansoni*) should also be considered.

**Village-level characteristics that promote the distribution of schistosome eggs also promote human and bovine infections in areas where schistosomiasis has reemerged.**

This research identified two such characteristics that place areas at risk of human schistosomiasis: bovine density and the use of night soil as an agricultural fertilizer. Infection prevalence varied greatly by village but few individual-level predictors of infection were identified in either endemic or reemerging areas (Chapters 2 and 3), providing further evidence that infection risks may be differentiated by local environments. Mathematical models have previously demonstrated the importance of internal potential – conditions that favor the schistosome life cycle – in dictating the long-term stability of schistosomiasis in a village [18,19]. The findings presented in Chapter 4 suggest a village’s internal potential may also determine its risk of schistosomiasis reemergence. Given the low infection intensities observed in the reemerging region, villages where large quantities of human waste are used to fertilize crops or the number of bovines per household is high may be primed for reemergence, providing crucial links in the schistosome life cycle that can promote *S. japonicum* infections if the parasite is reintroduced. The generalizability of the concept of internal potential to other parasitic infections warrants evaluation. In the broadest sense, the importance of local environments is obvious for most parasitic infections. For example, malaria requires mosquito habitat, and lymphatic filariasis requires both mosquito habitat and sufficient human-mosquito contact to allow for high biting rates. Rigorous evaluation of the local factors that sustain transmission for multiple parasites could advance understanding of the environmental determinants of human infections, and allow for interventions targeting multiple diseases.

The role of village-level characteristics in schistosomiasis reemergence has important implications for epidemiological analyses of schistosomiasis. Epidemiological analyses often focus on individual-level risk factors, but I found no association between household-level night soil use and *S. japonicum* infection. Instead, infection risk was determined by the average fertilization practices of all other households in a village. When risk factors for schistosomiasis or other environmentally mediated infections are examined, the definition of each risk factor must be carefully considered and the potential importance of ecological variables should not be ignored. As seen here, an individual’s infection risk may be determined not just by individual characteristics, by the behaviors of neighbors and by community-wide characteristics.
Surveillance strategies that rely on the detection of S. japonicum infected snails or the presentation of acute cases of schistosomiasis grossly underestimate the extent of schistosomiasis reemergence.

Surveillance methods that can accurately identify areas where schistosomiasis has returned in a timely manner are needed to enable prompt interventions to halt renewed transmission and to contain the spread of infections. Direct testing of human populations appears to be the most accurate indicator of reemergence, but human infection surveys can be costly and labor intensive. Targeted sampling of individuals at high-risk of infection, identified based on demographic or geographic characteristics, can allow for more efficient surveillance. Middle aged adults were identified as possible targets in Chapter 3. In Chapter 4, village characteristics were identified that can be used to define high-risk populations. High-risk populations could also be defined based on patterns of water contact. In endemic areas, spatial [20] and temporal [21,22] variations in water contact have been associated with schistosomiasis infection risk, as have particular water contact activities [23,24]. Little is known about the acquisition of S. japonicum infections in reemerging areas. An examination of high-risk water contact behaviors, including water contact timing, activity and spatial distribution is warranted in regions where schistosomiasis has reemerged.

Surveillance methods that rely on environmental sample collection are appealing as they can be less disruptive to residents than human infection sampling (which, in the studies described here, required three fecal samples from each individual). But in the present study, surveys for S. japonicum infected snails, had low sensitivity. Surveys for the presence of snails had higher sensitivity but the specificity of the method was below 50%. Surveys for schistosome-infected snails are a central element of schistosomiasis surveillance measures in China and are recommended for regions worldwide that are nearing the elimination of transmission [7,25]. Based on the evidence presented here, their use should be reconsidered.

Latrine effluent may offer an alternative sampling medium, as it is an indirect means of assessing human stool for S. japonicum eggs. Because egg output may be low and infections limited in post-control areas, samples from multiple latrines could be combined for analysis. Pooled sampling methods should be considered when monitoring for reemergence, as they can increase the probability of detecting S. japonicum while reducing the number of tests required. Batch analysis of mosquitoes for filarial infection using PCR has been used in lymphatic filariasis monitoring programs [26]. More research is needed to evaluate the feasibility and performance of this technique.

Schistosomiasis-induced liver fibrosis is slow to reverse and may even progress following treatment, highlighting the importance of infection prevention.

In Chapter 2, ultrasound-detected liver fibrosis was associated not only with current infection intensity, but with prior infection intensity despite prompt treatment of infections. Praziquantel targets the adult schistosome, but eggs may remain trapped in human tissue. The trapped eggs may continue to trigger inflammation and fibrosis, even after treatment, as suggested by work presented here and elsewhere [27-29]. This evidence, in combination with the limited reversal of severe fibrosis suggests infection prevention may yield greater improvements in health than regular treatment with praziquantel. To date, treatment of schistosomiasis has been the focus of disease control efforts rather than infection prevention.
Ultimately, control programs that reduce the prevalence and intensity of infections will reduce the number of new infections. But direct infection prevention measures, through environmental alterations or efforts to reduce high-risk water contact, should be evaluated for maximum reductions in morbidity.

**Implications for emerging threats, including climate change and natural disasters**

The latest climate change estimates suggest changes in temperature and rainfall may alter the geography of schistosomiasis, leading to the emergence of schistosomiasis in areas previously naïve to the parasite [30]. Warming temperatures may impact the range of the snail host and the parasite, whose maturation within the snails is temperature-dependent [31,32]. Less attention has been paid to the impact of hydrological changes on transmission or the potential for changes in both temperature and rainfall to alter crop selection and therefore human water contact. Natural disasters can also present schistosomiasis risks, as demonstrated by the 8.0 magnitude earthquake that struck Sichuan in May 2008, destroying water and sanitation systems and forcing movements of large populations between schistosomiasis endemic and controlled areas [33]. In fact, a relocation camp for earthquake refugees was located in one of the three reemerging counties described in Chapter 3. Local public health authorities were concerned about further schistosomiasis reemergence given the potential for displaced humans and domestic animals, as well as relief workers, from endemic areas to introduce *S. japonicum* to areas where schistosomiasis was controlled, potentially high levels of contact with irrigation and stream water, and the limited availability of sanitary toilets in refugee camps in the immediate aftermath of the earthquake.

Responses to climate change, natural disasters and other phenomena such as armed conflict, that threaten to increase schistosomiasis incidence can be formulated with the findings presented here in mind. Characterization of new infection risks must consider the importance of local environments in schistosomiasis transmission. For example, in regions where snail habitat faces climate-driven expansion, areas where the distribution of human and bovine waste is high, due to inadequate sanitation, fertilization practices or domestic animal ownership, may face the greatest risk of schistosomiasis reemergence or emergence. The importance of local environments also presents opportunities to mitigate infection risks, through interventions to alter local internal potential. Surveillance directed at high-risk populations in high-risk regions can allow for timely response to schistosomiasis emergence or reemergence.

**Recommendations**

Areas where schistosomiasis has been controlled are at risk of schistosomiasis reemergence, as documented by this study. With this threat in mind, a practical, highly sensitive post-control surveillance strategy is urgently needed. Current methods that rely on the reporting of acute schistosomiasis cases and detection of *S. japonicum*-infected snails are inadequate. Routine testing of high-risk human populations, such as middle aged adults, or residents of villages with high bovine densities or extensive night soil use, should be considered. Further research is needed to refine the definition of high-risk populations and to identify regional variations in infection risks. In addition, alternative surveillance strategies should be explored, including sampling of latrine effluent for *S. japonicum* eggs.
Schistosomiasis interventions should be considered with long-term suppression of transmission in mind. Bovine infections and night soil use appear to be important intervention targets. Environmental modifications have recently been included in some schistosomiasis control programs in China [34], but such programs have not addressed night soil use directly. The surprising finding that biogas was not associated with a decrease in infection risk warrants further evaluation, as installation of improved sanitation is being adopted as a schistosomiasis control strategy in some regions. Experimental studies are needed to evaluate the ability of field-constructed biogas systems to destroy schistosome eggs. Community-based randomized controlled trials of biogas systems may also be warranted.

Conclusions

There is a tension in infectious disease control, particularly the control of neglected tropical diseases that lack vaccines, between quick, inexpensive medical-based treatment measures and expensive, long-term environmental modifications to disrupt transmission. The research presented here suggests environmental determinants of schistosomiasis transmission must be considered for the long-term success of schistosomiasis control programs. Schistosomiasis is a disease of poverty, a disease that infects the rural poor and can reinforce poverty [35]. Even within the poor, rural communities presented in Chapters 2 and 3, socio-economic differences in infection prevalence were observed, as adults with the least formal education were most likely to be infected. Regions impacted by schistosomiasis often have limited resources to implement control initiatives, making inexpensive chemotherapy appealing. But reductions in infections may be transient if chemotherapy is not supplemented with other interventions and with effective surveillance measures. In order to attain sustainable reductions in schistosomiasis infections and morbidity, and to avert large-scale reemergence, governments and donors must be motivated to couple treatment with surveillance and alterations to local environments in high-risk areas.

References


